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NOTES ON THE MUSCULATURE OF THE MALE GENITALIA OF *HAEMONCHUS CONTORTUS*

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In connection with certain unpublished studies dealing with gametogenesis in *Haemonchus contortus,* mass sectioning of material under study revealed several longitudinal sections in dorso-ventral sequence, of the posterior region of the male worm. While the anterior portion appears slightly below the level of the more posterior region and the whole section is slightly tilted toward the left (thus rendering structures on the right more prominent to the observer) nevertheless, an unusually good picture of the organs involved is presented. Cross sections through this region from the posterior intestinal glands to the cloaca inclusive were photographed. These show the relationship of the organs herein described.

Fig. 21, Plate V is a photomicrograph showing the position of the spicules upon extrusion. It is here seen that the anterior portions of the spicules are in juxtaposition with the posterior intestinal glands (I. Gl.). Such a position of the spicules would place that portion of the spicules as indicated by the arrow, Fig. 7, Plate II, very near the opening of the intestino-germinal canal shown by the arrow in Fig. 20, Plate IV, formed by the junction of the vas deferens and intestine as shown by the arrow in the sagittal sections, Fig. 22, Plate V. The sections were cut at seven microns and stained in Heidenhain's iron hematoxylin.

The muscles involved in the retraction and extension of the spicules and the operation of certain bursal rays together with the location of glandular structures along the intestine and also the position of the posteriad region of the vas deferens were photographed. Camera lucida drawings were made, and the above-mentioned structures are herein briefly described.

Fig. 1, Plate I, shows the gubernaculum and the dorsal ray. The gubernaculum is strongly supported and held in place by longitudinal and transverse muscles as shown in Fig. 11, Plate III. Springing from each side of the gubernaculum is a thin cuticular structure which completely encircles the spicules and separates them from the intestine.

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Fig. 2, Plate I, shows a more ventral section. Here is seen a portion of the gubernaculum and the spicules faintly defined on each side of and anterior to the gubernaculum.

Fig. 3, Plate I, depicts a further ventral section just below the gubernaculum. The right spicule is more prominent than the left. The thickened recurved shanks of the spicules slightly overlap. The vas deferens appears near the bulge of the shanks of the spicules and proximal to the grooves which characterize the shanks. The spicules terminate in a heavily muscled sheath.

Fig. 4, Plate I, is a still more ventral section. Portions of the heads of the spicules are visible together with the muscles which operate the spicules. Parts of the cloacal muscular system and one pair of glands situated anterior to the cloaca are seen.

In Fig. 5, Plate I, the cloacal dilator muscles are more evident, and posterior to the gland described in Fig. 4 there may be seen two additional glands.

Fig. 6, Plate I, shows the cloacal dilatory muscles and the intestine midway between these muscles. The anterior portion of the intestine is constricted, whereas the more posterior portion is dilated and empties into the cloaca wherein may be seen the ends of the spicules.

Fig. 7, Plate II, is a camera lucida drawing of the section appearing in Fig. 4 to which were added the essential structures appearing in sections 2, 3, 5, and 6, and affords a basis for the interpretation of the function of the structures already presented. These functions are defined primarily on their various anatomical locations, origins, and insertions.

The origins of muscles 1 and 3, *Retractor spicularis lateralis* (1 and 3), could not be definitely determined, but apparently they arise laterally from the body walls of the worm. They are inserted on the heads of the spicules and their function is to retract the spicules.

Muscle 2, *Retractor spicularis centralis* (2), apparently arises from the dorsal wall of the body of the worm and proceeding caudally bifurcates into two smaller portions which insert on the heads of the spicules. Its function is to retract the spicules.

Muscle 4, *Protractor spicularis,* and its opposite fellow running from the heads of the spicules to well within the bursal lobes arises from the inner walls of the rays of the lobes and proceeds cephalad. It receives two muscular serrations arising laterally from the wall of the body before it inserts along the lateral anterior one-third of the spicule and on the head of the spicule. Its function upon contraction is to cause protrusion of the spicule and probably assists in contraction of the bursa. The caudal ends of the spicules are encased in a cuticular and heavily muscled sheath. This latter seems also to include ventrally the terminal portion of the conventionally defined cloaca.

Muscle 5, *Dilator cloacae,* and its fellow on the opposite side arise from the walls of the body and insert on the cloaca, the cuticular body wall and spicule sheath. The function of these two muscles is to dilate the cloaca and the cloacal aperture.

Muscle 6, *Bursa expansa,* lying along the floor of the caudal wall of the worm arises from the rim of the cuticular opening of the cloaca and inserts on the lateral body wall and the inner rim of the exterior wall of the bursal rays. Its function is to expand the bursa.

It seems, from a study of the materials here presented, that there are more than one of the muscles 5 and 6 as these muscles or remnants of these muscles appear in five different sections.

Fig. 9, Plate II, is a photomicrograph × 900 of the thickened shank and posterior portion of the spicules as seen in Fig. 3. Within the grooves of the shank are the sperms enclosed in their gelatinous envelopes.

The camera lucida drawing, Fig. 8, including the same sectional area as that shown in Fig. 9 shows in addition a portion of the vas deferens from which a sperm has been dislodged.

The sperms lying within the grooves of the spicules were seen in sections on other slides. In one particular instance the spicules were not cut by the sectioning knife but were entirely displaced from their surrounding structures, revealing a clearer picture of the position of the sperms than the one here presented. Cross sections through the anterior rectal glands (Figs. 10 and 11, Plate III) show the muscular support of the gubernaculum and the anatomical relationship of the gubernaculum, spicules, spicular sheath, vas deferens and intestine. In a series of cross sections, Figs. 12 to 20, Plate IV, inclusive, the intestine and vas deferens evidently approach each other and join to form one canal. The lumina of these respective tubes are almost obliterated by the intimate structure of the intestinal glands which empty into the resulting canal which proceeds caudad and empties directly into the cloaca (see arrow, Fig. 20). It is at this point that sperms pass into the spicules.

DISCUSSION

Looss (3) describes three pairs of muscles of the guiding-piece (gubernaculum) and concludes his description thus: "Comparing now the various effects just described as probably produced by the six muscles of the guiding-piece it becomes obvious that by their help the tip of the latter is enabled to move, within certain limits of course, in every direction, a capability which is no doubt of importance during copulation." Similarly placed muscles are described by Looss for *Ancylostoma duodenale.* There is considerable evidence in this study, Plate III, Fig. 11, that three pairs of muscles are intimately connected with the gubernacu-

lum which could produce slight movement in three directions; namely, a cephalad-posteriad, dorso-ventral, and a lateral direction. These muscles, however, could not be traced in entirety in the material available.

The insertion of muscle 5 and the entire muscle 6, Plate II, correspond to certain muscles of the genital cone as described by Looss (3). The more anterior structures lying along the spicular sheath described in Fig. 4, Plate I, as glands are described by Looss (3 and 4) as muscle cells which give rise to rectal, sphincter muscles.

No muscle fibers arising from these structures could be discerned in this study and since they are intimately connected with the lumen of the intestine they are designated rectal glands.

Muscles 1 and 3, Plate II, are described by Looss (4) under the caption *Musculi retractores.* Muscle 2, Plate II, was not observed by Looss. Muscle 4 and its opposite fellow are described as *Musculi exsertores spiculorum.* Their descriptions in general parallel the description here given with the exception that they are located by Looss (4) as originating in the crest of the cloacal wall near its anterior end instead of well within a bursal ray. The latter description would serve to assist in contracting the bursa before the spicules are extruded through the anal aperture.

Looss (4) also observed sperms on the flattened side of the spicules between the two longitudinal crests. He explains the presence of the sperms in the canal formed by the proximity of the spicules as being sperms which were not all carried out during copulation and upon retraction of the spicules were drawn back into the spicular sheaths. He further excludes the possibility that they made their way there from the point where the ductus ejaculatorius enters the cloaca. It is here suggested that in *H. contortus* the sperms enter the spicules directly from the terminus of the intestino-germinal canal and are carried in the grooves of the spicules to the vagina of the female during copulation. It would be difficult for the sperms to gain their position in the grooves of the spicules by other means since the spicules pass through an unperforated cuticular canal and are closely applied to each other upon their emergence into the cloaca. It is conceivable, however, that the spicular apparatus can be moved into a position most favorable for the reception of the sperms between the spicules. This position is well illustrated in Plate V, Fig. 21. Here the anterior end of the spicules approximates the glandular region of the rectum and brings the anterior grooved portion of the spicules opposite to the opening in the intestino-germinal canal.

SUMMARY

A study of longitudinal and cross sections through the posterior region of the male nematode, *H. contortus,* shows:

That the gubernaculum is supported and slightly activated by longitudinal and transverse muscles and that the location of these muscles implies that slight movement is possible in three directions.

Other muscles are described and their functions are explained on the basis of their origins, insertions, and locations.

a. Muscles 1 and 3, each named *Retractor spicularis lateralis,* function—to retract the spicule.

b. Muscle 2, *Retractor spicularis centralis,* function—to retract the spicule.

c. Muscle 4, *Protractor spicularis,* function—to extrude the spicule and assist in contraction of the bursa.

d. Muscle 5, *Dilator cloacae,* function—to dilate the cloaca.

e. Muscle 6, *Bursa expansa,* function—to expand the bursa.

Upon extrusion of the spicules the anterior portions of these organs wherein are the origins of the longitudinal grooves, assume a position opposite the break in the wall of the intestino-germinal canal and here receive sperms passing down through this common canal from the vas deferens.

Spermatozoa contained in gelatinous capsules are found in the grooves formed by the longitudinal spicular ridges.

The spicules conjointly act as a penis to transfer the spermatozoa from the male into the vagina of the female during copulation.

Two to three pairs of intestinal glands are present proximal to the intestine.

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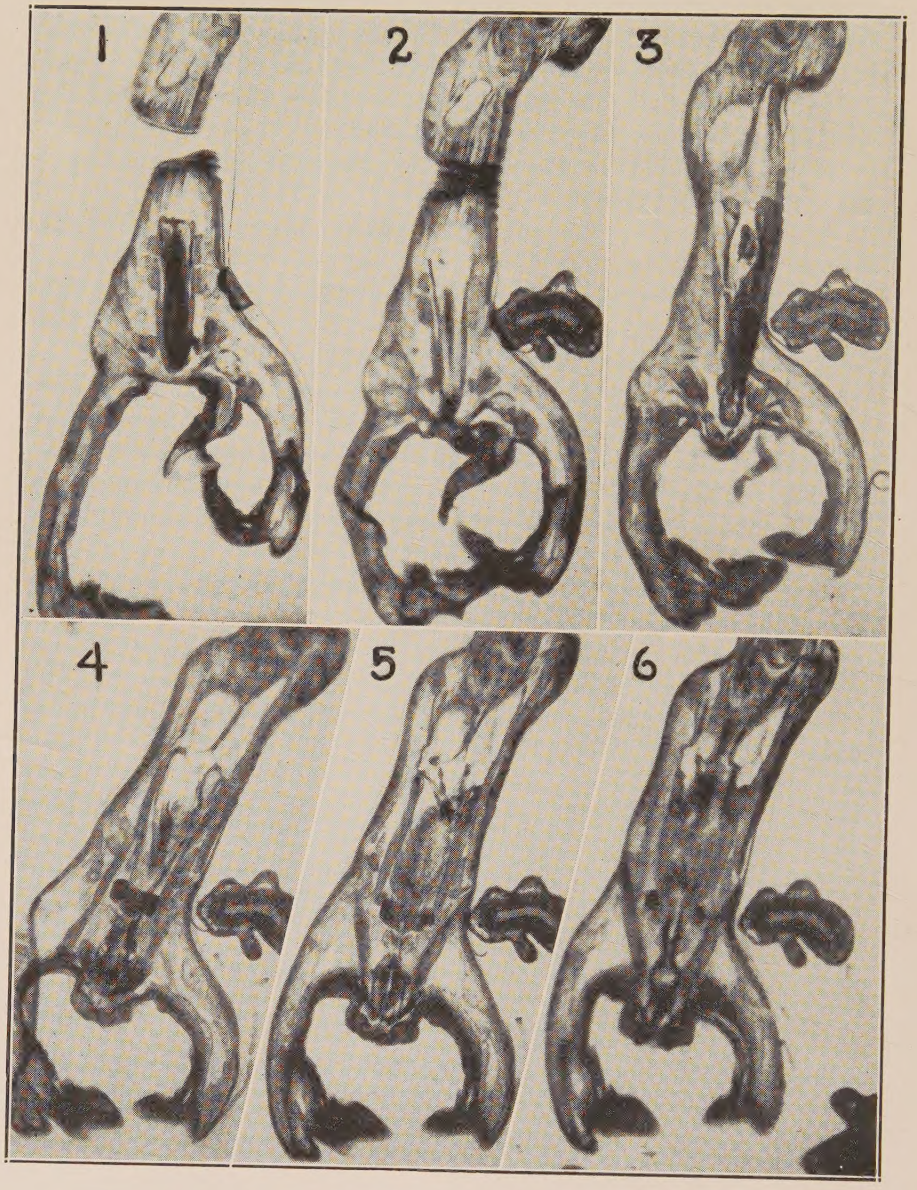

PLATE I

FIGS. 1–6. Photomicrographs × 100 of dorso-ventral sections in series through posterior portion of the male *Haemonchus contortus*, showing location of organs described.

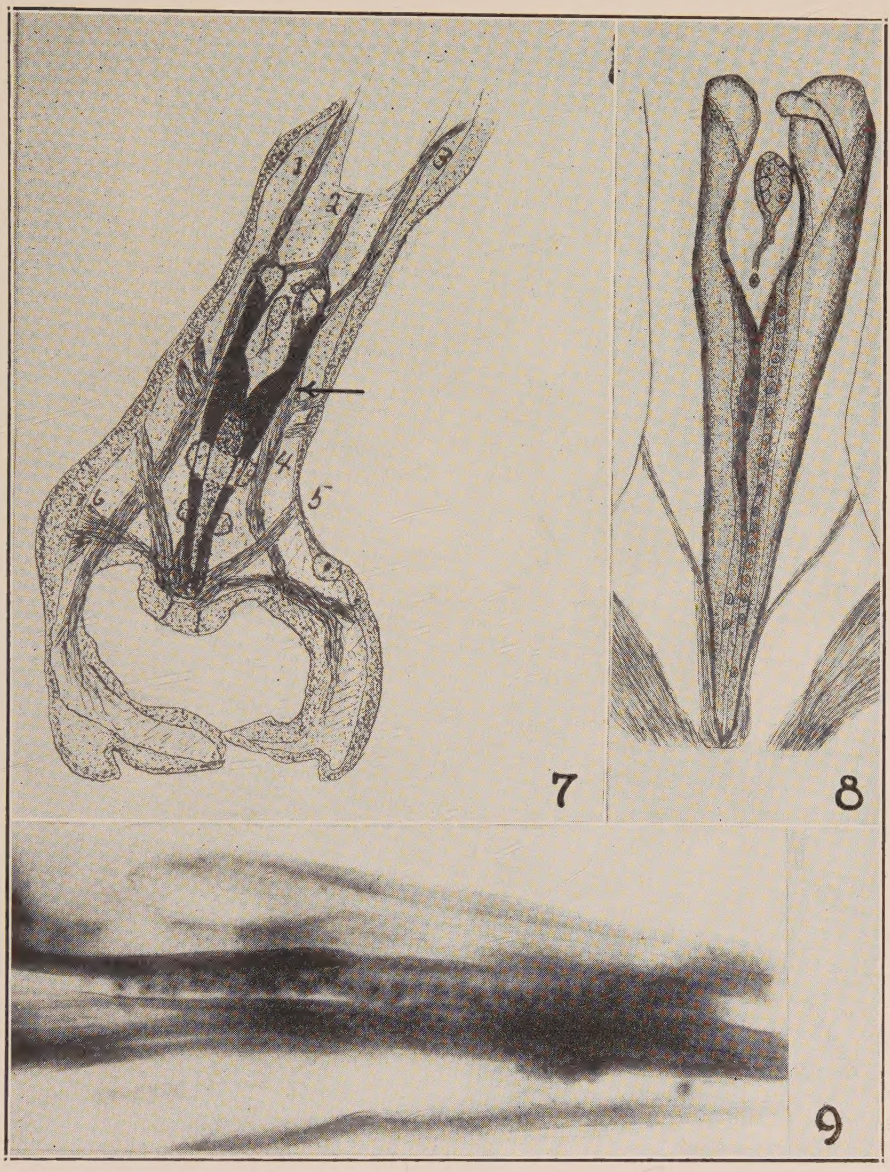

PLATE II

FIG. 7. Composite camera lucida drawing ×150, reduced to 5/6 original size, of the essential structures appearing in Figs. 2–6, inclusive.

FIG. 8. Camera lucida drawing ×333, reduced to 1/3 original size. Same section as photomicrograph, Fig. 9.

FIG. 9. Photomicrograph ×900 of a portion of the spicules showing sperms in the grooves of the spicules.

PLATE III

FIG. 10. Photomicrograph ×900. Transverse section through the posterior portion of a male *H. contortus*. The large black structure in the lower left hand corner of the picture is the gubernaculum. There follow in sequence the spicules, the intestine, and the glandular vas deferens.

FIG. 11. Same section as shown in Fig. 10 in slightly different plane showing support of gubernaculum by transverse and longitudinal muscles.

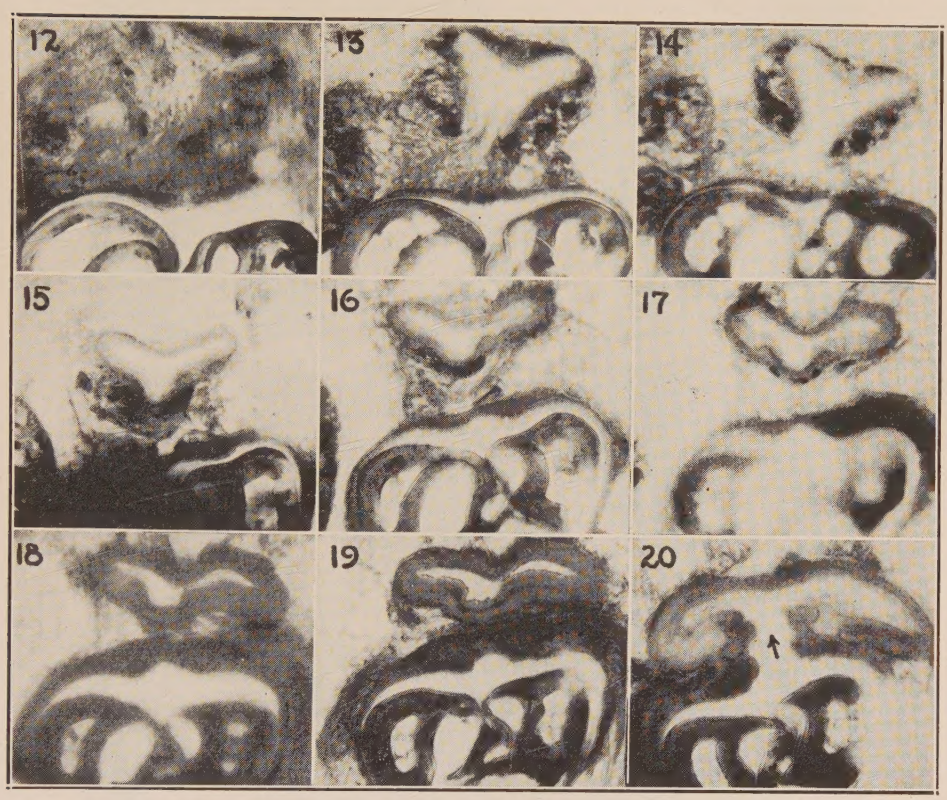

PLATE IV

FIGS. 12–20. Photomicrograph ×810. Transverse sections in series from the region of the posterior intestinal glands to the cloaca showing the highly glandular portion at the mergence of the intestine and vas deferens and the connection of the resulting canal with the cloaca.

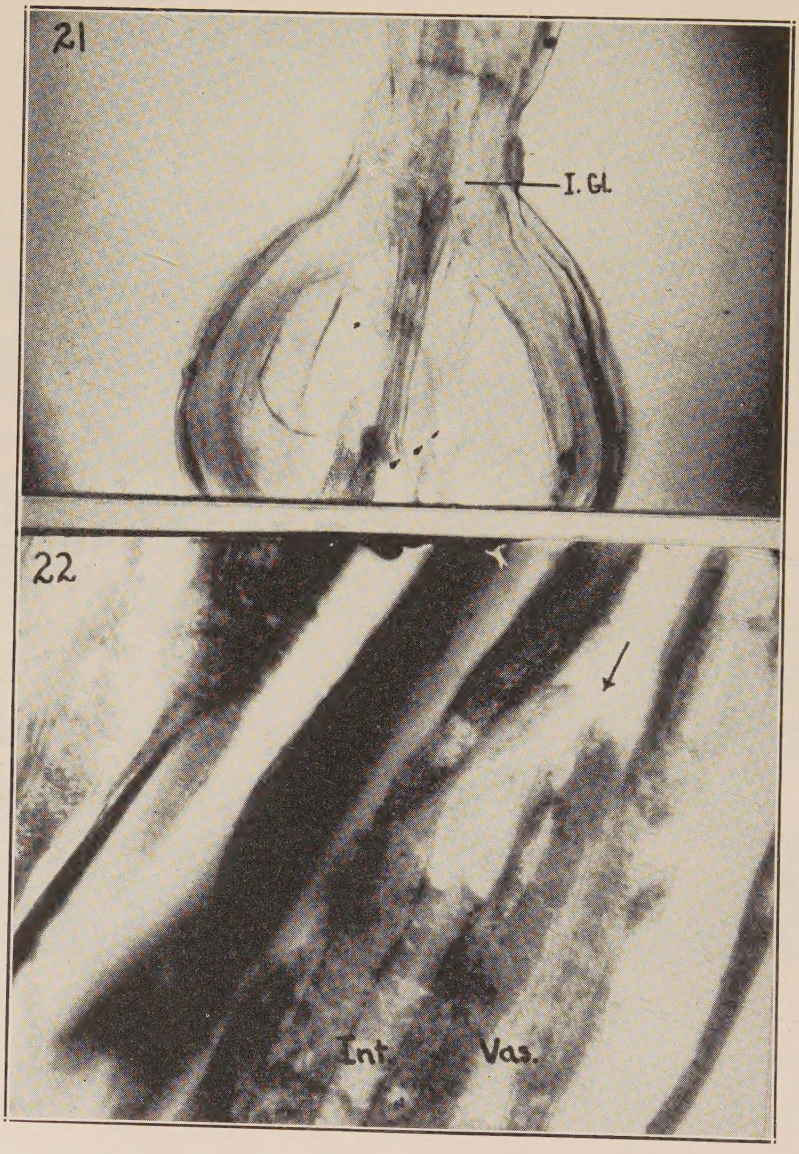

PLATE V

FIG. 21. Photomicrograph × 100 showing position of spicules upon extrusion.

FIG. 22. Photomicrograph × 900 of sagittal section showing portion of intestino-germinal canal formed by the intestine (Int.) and the vas deferens (Vas.).

SIPHONAPTERA: *PTILOPSYLLA DUNNI,* A NEW SPECIES OF BAT FLEA FROM PANAMA[1]

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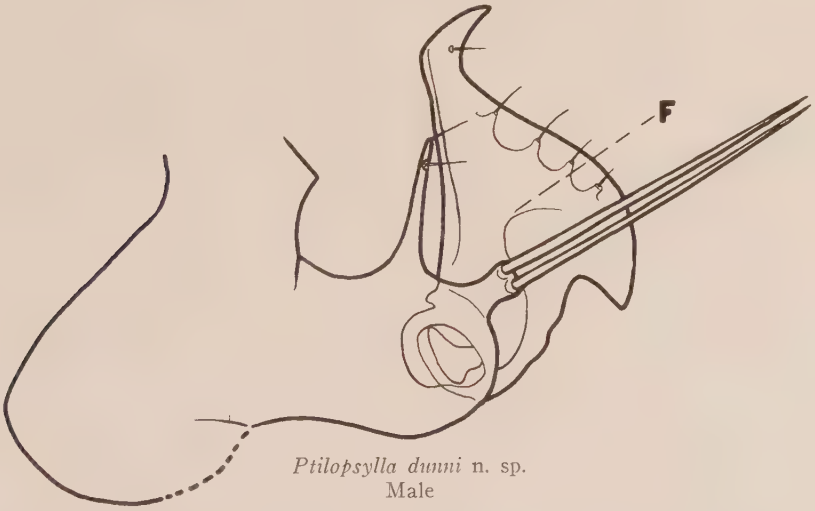
Among materials recently received for determination from the American Museum of Natural History through the courtesy of Dr. C. H. Curran, were 3 males of the genus *Ptilopsylla* Jordan and Rothschild, 1921,[2] collected from a bat, *Molossus coibensis* Allen, Panama City, December 6, 1933, by L. H. Dunn. The specimens were thought to represent a new species and one was sent to Dr. Karl Jordan, of the British Museum, for comparison with the only previously known species of the genus, *Pt. leptina* Jord. and Roths., 1921, which was described from a single male specimen collected from a bat at Santissima Trinidad, Asuncion, Paraguay. The writer's opinion was confirmed and Dr. Jordan stated that in the British Museum there are a male and female apparently from the same lot. Comparative notes kindly furnished by Dr. Jordan are incorporated in the description which follows.



Ptilopsylla dunni n. sp.
Male

Head: Frontal tubercle small but distinct. The two oral spines narrow, posterior one longer and broader than anterior one. Frons with an anterior row of ten bristles, the upper one longest and situated near anterior margin of antennal groove; lower bristle shorter than upper bristle but much longer than intervening ones. Ocular bristle, situated just above vestigial eye at anterior margin of antennal groove, long, reaching well beyond apex of genal process. Two other prominent bristles on frons; one near anterior margin of antennal groove between ocular bristle and upper bristle of anterior row of frons, other above base of posterior oral spine at level of frontal

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[1] Contribution from the Rocky Mountain Laboratory (Hamilton, Montana), of the Division of Infectious Diseases of the National Institute of Health.

[2] Ectoparasites 1: 158–162.

tubercle. Occiput with numerous rather stout bristles. Posterior margin antennal groove bordered with about 10 small bristles. Genal process acuminate; tip rounded off. Maxillary and labial palpi equal in length and slightly less than half length of fore coxa.

Thorax: Pronotum with comb of twenty-two curved spines; also two rows of bristles and a few scattered additional bristles. The mesopleura bear about 15 bristles. Rod dividing mesopleura in other genera obsolete but represented by remnant projecting dorsally from bar separating mesopleura from mesosternum. Metepimeron bears 14 or 15 bristles. Metanotum with two short spines on each side.

Abdomen: Tergites and sternites with strong transverse incrassations. Incrassation of basal sternite short; that of sternite VII broad, Y-shaped in lateral view. Tergite I with comb of about 8 spines, dorsal ones of which are two-thirds as long as dorsal pronotal spines. The two lower bristles of tergites II to VII widely separated from other bristles. Sternite VII has 4 or 5 bristles on each side. One antepygidial bristle on each side.

Modified segments: Tergite VIII bears about 7 bristles above stigma including long bristle situated at about upper fifth of apical margin. Sternite VIII long, broad basally, narrowing gradually toward apex, upper margin nearly straight. On outside along ventral margin about 15 bristles of size found on eighth tergite; on inside a row of very closely set bristles, curved ventrad, each ending in fine wavy filament. Longer bristles are near apex and longest is about length of hind tibia. Ventroproximal margin of this sclerite is heavily sclerotized and extends to incrassation of seventh sternite. Manubrium of clasper short and broad. Clasper with two processes: upper one slender, pointed, conical, with an apical bristle and another situated subapically; lower process much shorter, truncate, somewhat dilated apically and bearing two long, strong bristles. Exopodite (F in Fig.) broad proximally, ventral margin deeply notched, lower or posterior angle pointed, apical margin rounded and upper or anterior angle drawn out into posteriorly directed pointed hook. Apical portion of ninth sternite large, conical; posterior margin slightly incurved twice, a bristle situated distally to the proximo-ventral angle and another further distad, but before the middle.

Length: Holotype, 1.8 mm (exclusive of sternite VIII).

Host: Bat, *Molossus coibensis* Allen.

Locality: Panama City, Panama.

Holotype: Male, in collection of Rocky Mountain Laboratory.

Paratypes: One male, deposited in British Museum; one male in American Museum of Natural History, New York City.

According to information received from Dr. Jordan the female agrees with the male apart from the sexual differences but has the sclerotizations of the head, thorax and abdomen less pronounced.

The species is very similar to *Pt. leptina* from which it may be most readily distinguished by the shape of the exopodite. In *leptina* the exopodite is described as "large, resembling a short-stalked mushroom; it is proximally narrow and soon widens out into a broad hood; the apical margin is rounded, the ventral (= distal) margin below the hood is deeply incurved, the anterior margin almost straight. The anterior (or upper) corner of the hood is truncate-rotundate; the posterior (or lower) corner, which is much obscured in the specimen, appears to be broadly truncate, as indicated in the figure." Also, in *dunni* tergite VIII bears about 7 bristles above the stigma including the long bristle situated at about the upper fifth of the apical margin; in *leptina* there are 11 and the long bristle is situated at about the upper third. The mesopleura bear about 15 bristles while in *leptina* there are 22 or 23.

STUDIES ON PHENOTHIAZINE. XI. THE EXCRETION OF PHENOTHIAZONE*

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In recent years the status of phenothiazine has changed from that of a laboratory curiosity and theoretical precursor of the thiazine dyes to that of a compound with important insecticidal properties. More recently the compound has demonstrated its usefulness as a bactericidal agent in the treatment of urinary tract infections, as a fungicide, and as an anthelmintic. The oxidation products of phenothiazine are the sulphoxide, phenothiazone and thionol. Under appropriate conditions the leuco bases of the two latter compounds can be formed. Exposure of phenothiazine to air, moisture and light results in oxidation to the phenothiazone stage at least. Oxidation in the animal body occurs with even greater ease. Since some of the interesting properties of phenothiazine may be associated with the oxidation products it is desirable to record information pertaining to the fate of phenothiazine in the animal body.

In a previous paper on the fate of phenothiazine it was reported by DeEds, Eddy and Thomas (1) that the gastric administration of phenothiazine to rats, rabbits and humans resulted in the urinary excretion of phenothiazine in some water-soluble form, and of the reversible oxidation-reduction system thionol-leucothionol. These authors also stated, "In a personal communication, containing a report on the examination of a sample of the precipitate obtained from a phenothiazine urine, Mr. Wheeler of the Food Research Division, U. S. Department of Agriculture, has stated that the precipitate was largely phenothiazine plus a small amount of phenothiazone." However, it was impossible, at that time, to obtain additional evidence which was regarded as proof for the excretion of phenothiazone. This fact together with the statement in the literature (2) that phenothiazone and its leuco base are insoluble in water led to the conclusion that appreciable quantities of phenothiazone and its leuco base did not occur in the urine.

The fate of phenothiazine in sheep has been studied by Collier (3) who reported that the oxidation product was phenothiazone, excreted almost entirely in the form of the ethereal sulfate of leucophenothiazone. Subsequently an examination of the urine of sheep dosed with pheno-

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thiazine was also made by Lipson (4). In commenting on the report by DeEds, Eddy and Thomas, Lipson stated, "Their results, however, cannot be accepted as conclusive, as they were unable to isolate a crystalline product or derivative of thionol, and were forced to rely upon the method of oxidation-reduction potentials in order to characterize their product. As phenothiazone, another oxidation product of phenothiazine, will also give rise to a reversible oxidation-reduction system, this compound may have been present in the urine examined by these workers."

This statement implies that determination of the potential of a reversible oxidation-reduction system and comparison of the potential with that of a system of known identity are not reliable criteria for establishing the identity of the system in question. The further implication seems to be that the method of potential measurement would not have permitted the differentiation of the two systems phenothiazone-leucophenothiazone and thionol-leucothionol.

Because the measurement of oxidation-reduction potentials, where such measurements are possible, is not an orthodox procedure for identification purposes, justification for its use merits some discussion. The usual procedure of identification by melting point and mixed melting determinations represents an identical behavior of a known and unknown system at one point. The plotting of the oxidation-reduction potential of a known system, having a ratio of reductant to oxidant equal to 1, against variations in pH, and the superposition on the resultant curve of potential values similarly obtained on an unknown system represents an identical behavior of the two systems at many points. Since the changes in slope of the curve at certain pH values are due to the presence of ionizable groups, the chances that superimposable curves represent different compounds must be quite remote. The possibility of confusing the system thionol-leucothionol having two hydroxyl groups in the leuco form with the more positive system phenothiazone-leucophenothiazone, having one hydroxyl group in the leuco form, is negligible. Evidence that the phenothiazone-leucophenothiazone system is excreted in the urine of rats, rabbits and man, and is more positive than the thionol-leucothionol system will be submitted below.

The following quotation from the paper by DeEds, Eddy and Thomas (1) contains the evidence, at that time not recognized, that phenothiazone and particularly its leuco base occur in the urine of rats, rabbits and humans receiving phenothiazine: "The combined dilute alcohol solutions, after removal of the phenothiazine, were found to contain a considerable amount of the leuco base of the red dye, as indicated by results of aeration, addition of hydrogen peroxide, alkalinization, or oxidation facilitated by passage through a filter paper, all of which yielded a deep red solution, extractable with chloroform, as already described. Extraction of the

combined dilute alcohol solutions with ether in five-foot liquid extractors of about one liter capacity yielded a straw-colored ether extract. After spontaneous evaporation of the ether, the residue was extracted with boiling carbon tetrachloride and filtered while hot. The filtered carbon tetrachloride solution was chilled in an ice bath and evaporated to a small volume under a current of air. Fine needle crystals of an almost colorless product separated out, and were sucked dry on a micro suction filter. The product was dried in an Abderhalden dryer, and the melting point determined. The nearly colorless crystals slowly underwent spontaneous conversion to a dark red powder. The melting point of the crystalline product was found to be 172 to 174° C."

Before submitting proof that the crystalline product melting at 172–174° C is leucophenothiazone an error in the report by DeEds, Eddy and Thomas (1) should be pointed out. It was concluded that the product was probably the leuco base of thionol. If the potential of the system represented by the crystalline product melting at 172–174° C and its oxidant had been studied this error would not have been made. Likewise, if a sample of pure phenothiazone had been available at that time, a melting point determination of its leuco base would have been made and the error also avoided in that manner. Attempts to prepare phenothiazone at that time and for that purpose were unsuccessful.

Following the report by Collier, a sample of phenothiazone was obtained from that author, and additional quantities were prepared by the method of Pummerer and Gassner (5). Each of these products was examined as follows: The phenothiazone was dissolved in a small volume of 95 per cent ethyl alcohol, distilled water was added and the mixture brought to a boil. A sufficient quantity of powdered sodium hydrosulfite was then added to the concentrated hot solution of phenothiazone to reduce it completely to the leuco base. Clouding of the hot solution occurred due to the lower solubility of leucophenothiazone and to small amounts of free sulfur. The mixture was then cooled under tap water whereupon a considerable amount of a fine white crystalline precipitate formed. The precipitate was removed by centrifuging. This precipitate was dissolved in hot carbon tetrachloride and the hot solution filtered. The carbon tetrachloride solution was placed in an ice bath and evaporated to a small volume under a current of air. Minute needle crystals of leucophenothiazone separated out. The crystals were nearly colorless, but en masse had a faint purple tinge, which may have been due to slight surface oxidation. On standing exposed to air for several hours the product darkened, a reaction which was greatly accelerated by ammonia gas. The leuco base prepared from the material furnished by Collier and that prepared in our laboratory had a melting point of 172–173° C, and the mixed melting point remained unchanged.

Samples of the product isolated from rat, rabbit and human urine as described by DeEds, Eddy and Thomas were again examined and melting point determinations made. The value of 172–173° C was obtained and remained unchanged when mixed with an equal weight of synthetic leucophenothiazone. Therefore, the product obtained from rat, rabbit and human urine, melting at 172–173° C and previously regarded as the leuco base of thionol, was undoubtedly leucophenothiazone.

As far as we know this is the first time that the melting point of leucophenothiazone has been reported. Attempts to isolate crystals of leucothionol by a similar procedure and determine the melting point have failed. The crystals have been obtained but the spontaneous oxidation of leucothionol proceeds so rapidly that melting point determinations have not been feasible. It is entirely possible that melting point determinations could be made if all operations were carried out in an atmosphere of nitrogen.

The fact that leucophenothiazone can be prepared, isolated, and the melting point determined in the presence of air whereas the same cannot be accomplished with leucothionol shows that the reversible system phenothiazone-leucophenothiazone has a potential appreciably closer to the oxygen electrode than that of thionol-leucothionol. Therefore, in a urine containing both these oxidation-reduction systems, the usual reducing properties of urine would keep the phenothiazone system in the leuco form for a longer period of time than would be the case with the thionol system.

After examining the various fractions prepared from the urine of sheep dosed with phenothiazine, Lipson (4) stated that Fraction 2 (b) yielded, after three recrystallizations from water, a red compound melting at 161–162° C, and was shown to be phenothiazone. Lipson further states, "a very small portion of Fraction 2 (b) was insoluble in boiling water, and could be recrystallized from aqueous alcohol to give a yellow solid which melted indefinitely at 170° C. This was probably some phenothiazine which had not been precipitated by acid in Fraction 1." However, since the melting point of leucophenothiazone is 172–173° C, there is a reasonable probability that the compound described by Lipson may have been leucophenothiazone.

SUMMARY

1. Evidence has been presented to show that the reversible oxidation-reduction system phenothiazone-leucophenothiazone, as well as thionol-leucothionol, occurs in the urines of rats, rabbits and humans receiving phenothiazine.

2. The validity of potentiometric identification of a reversible oxidation-reduction system has been discussed.

3. The melting point of leucophenothiazone has been shown to be 172–173° C. Samples of leucophenothiazone isolated from rat, rabbit and human urines were identified by their melting points, namely 172–173° C, and by their mixed melting points with synthetic leucophenothiazone.

4. Leucothionol oxidizes so rapidly that it has been impracticable to obtain its melting point.

5. It has been suggested that a compound isolated from the urine of a phenothiazine-dosed sheep by another worker was leucophenothiazone and not phenothiazine.

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PHYSIOLOGY OF A BACTERIA-FREE CULTURE OF *TRICHOMONAS VAGINALIS*. IV. EFFECT OF HYDROGEN ION CONCENTRATION AND OXYGEN TENSION ON POPULATION[1]

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PART I

EFFECT OF HYDROGEN ION CONCENTRATION ON POPULATION

No record of previous investigations of the physiology of *Trichomonas vaginalis* in bacteria-free culture has been found in the literature. Numerous workers have cultivated the organism in the presence of bacteria, and its response to the hydrogen ion concentration of various media has been recorded. Bland et al (1932) reported successful culture in media which shifted from pH 6 to pH 8 during the culture period "without any very noticeable effect upon the growth of the flagellates." Lynch (1922) maintained cultures in several media which "are slightly alkaline and become more so." Andrews (1929) used adjusted media varying from pH 7 to pH 8 and found an optimum growth at pH 7.6. Davis (1929) obtained successful culture "provided the pH was similar to that of human blood." It is difficult to determine to what extent these findings were affected by the bacterial flora. Differences in the culture medium used as well as in the nature and concentration of the bacteria present undoubtedly affect the rate of growth.

Other trichomonads have been investigated in bacteria-free culture. In a study of *Trichomonas foetus* Cailleau (1937) found an optimum pH between 7.0 and 7.6 with survival between pH 5.5 and 8.5. Riedmuller (1936) reported an optimum pH range of pH 6.5 to 7.5 for the same organism and Morisita (1939) found the optimum between pH 6.6 and 7.8 with survival between pH 5.6 and 8.4. Cailleau (1937) cultivated *Trichomonas columbae* in media ranging from pH 6.6 to 7.6 and found that the addition of calcium carbonate prolonged the life of the cultures.

The experiments reported in this paper were designed to determine the extremes of hydrogen-ion concentration which would support growth

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of the organism in the absence of bacteria and to discover the optimum pH for a medium which would maintain repeated subculture.

MATERIALS AND METHODS

The culture medium consisted of Difco liver infusion agar slants (3 ml) overlaid with 6 ml of modified Ringer's solution containing 5% of human serum. The Ringer's solution contained 0.6% NaCl and 0.01% $NaHCO_3$, KCl, and $CaCl_2$. The pH of both the agar slants and the fluid medium was adjusted with normal HCl or normal NaOH, after which they were buffered with 0.25% sodium phosphate. This concentration of buffer supported the maximum population with only minor changes in pH during the first four days of culture and was chosen after observation of cultures buffered with 0.1, 0.25, 0.50, and 0.87% sodium phosphate. The organisms were cultured in 13×120 mm tubes, plugged with cotton and incubated at $37.5 \pm 1°$ C. All manipulations of the organisms were made with cotton-stoppered pipettes, sterilized by hot air and controlled by mouth suction. The pH determinations were made with a glass electrode and a Cameron pH meter. Before each series of determinations the electrode was checked against buffers standardized at pH 4.0 and 7.38. One ml of culture fluid was withdrawn into a clean test tube and diluted with 2 ml of distilled water. After thorough agitation this fluid was transferred to the cup of the pH meter. The error due to discharge of carbon dioxide from the fluid was probably negligible, since the production of carbon dioxide was not demonstrable. In each case the pH of tube number 1 in each group was checked at each period of examination with that of the corresponding uninoculated control tube.

The populations were determined with a hemocytometer after 2, 4, and 6 days of incubation. Each count included 10 large squares having a total volume of 1.0 cu mm.

Aseptic precautions were observed throughout the experiments and at the conclusion of each series the cultures were checked for bacterial contamination by plating them on blood agar or chocolate agar. Less than one per cent of the cultures were found to be contaminated, and the data pertaining to these specimens are not included below.

The stock cultures originated from the bacteria-free culture isolated by Ray E. Trussell (1940) and were checked for bacterial contamination as reported by Trussell and Plass (1940).

An inoculum consisting of 0.2 ml of a uniform suspension of organisms combined from several four-day-old stock cultures was used in each experiment. Six tubes were inoculated at each of sixteen different reactions between pH 7.8 and 5.1. One uninoculated tube was maintained at each pH as a control. All cultures in each series were inoculated from the same suspension of organisms. It was therefore unnecessary to con-

sider the initial population as a variable factor. Series I and Series II are duplicates. The cultures in Series I were also subcultured on the fourth day.

RESULTS

Maximum populations in both series and in the subcultures were found on the acid side between pH 5.1 and pH 6.0. The growth optimum shifted with the time of incubation. In Series I, the optimum at 48 hours was between 5.25 and 5.4, at 96 hours between 5.5 and 6.0, while at 144 hours it dropped again to a range of pH 5.15 to 5.5 (Fig. 1). The

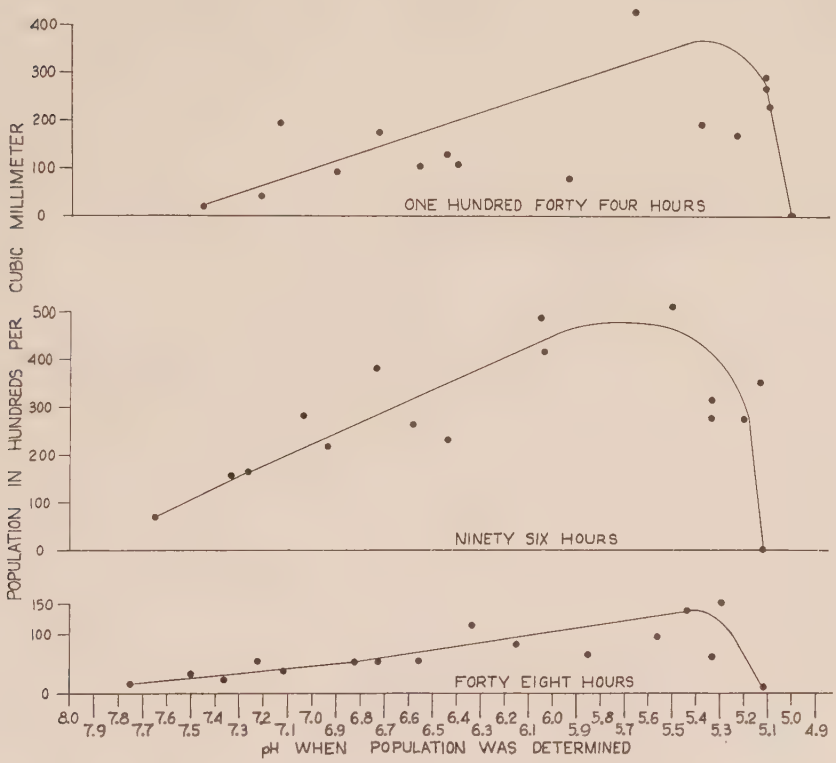

FIG. 1. Series I. Population plotted against pH. Curves fitted free hand.

optima of Series II approximated those of Series I as follows: At 48 hours, pH 5.45 to 5.55; at 96 hours pH 5.5 to 5.8, at 144 hours pH 5.15 to 5.55. Population values fell off sharply as the pH was reduced below the optimum, and multiplication ceased below pH 5.0 (Fig. 1). Broader ranges of pH were tolerated on the alkaline side of the optima with multiplication practically stopped between pH 7.55 and 7.75 (Fig. 1). Subculture was unsuccessful above pH 7.55.

Minor fluctuations occurred in the pH of both the uninoculated controls and the experimental cultures. Comparison of the pH of experi-

mental and uninoculated control tubes after the same intervals of incubation indicates that these changes in pH do not have a significant bearing upon the conclusions to be drawn from the data.

DISCUSSION

The correlation between these observations of a slightly acid optimum pH in bacteria-free cultures of *Trichomonas vaginalis* and the pH of the vaginal secretion in clinical cases of human trichomonas vaginitis has already been pointed out by Trussell and Plass (1940). Trussell and MacDougal (1940) employed a glass electrode technique in an investigation of the relation of vaginal acidity to various types of vaginal flora. In 18 out of 23 cases where vaginal trichomonads were observed they found an average vaginal pH of 5.58 with a range from pH 4.55 to 6.38. It is well known that introduction of acid solutions, such as dilute vinegar or acetic acid, are helpful in the treatment of trichomonas vaginitis. The very narrow range which has been observed to separate the optimum from the lower limit for multiplication on the acid side possibly demonstrates the mechanism behind this clinical observation.

There is apparently no record of other attempts to culture *Trichomonas vaginalis* in media adjusted to reactions well down in the pH scale and buffered to hold the reaction relatively constant. This in itself would account for the reports by other workers of an alkaline optimum. Bland et al (1932) started cultures at pH 6.8 and found that the pH shifted to the alkaline side in both experimental and control tubes. If they had started their cultures at a more acid reaction different conclusions might have been drawn. In addition to the possible effect of the presence of bacteria in cultures studied by earlier workers, one other factor should be considered. In any case where the pH of the culture medium was initially high and was allowed to drift down the scale and across an acid optimum the length of time elapsing before an acid limit would be reached would inevitably be longer with a higher total population than if the initial pH were lower. Thus if only the original pH were considered the optimum pH might appear to be at a higher level.

SUMMARY

1. Under the conditions of these experiments *Trichomonas vaginalis* exhibits a maximum population in an acid medium between pH 5.5 and 6.0.

2. Multiplication is limited or stopped by reactions below pH 5.0 and above pH 7.55.

3. There appears to be a correlation between these results and the conditions in the human vagina in cases of natural infection.

4. The use of acid solutions in the treatment of trichomonas vaginitis

is in harmony with the limited range between the optimum pH and the acid limit of multiplication.

Part II

EFFECT OF OXYGEN TENSION ON POPULATION

Riedmuller (1936) found the growth of *Trichomonas foetus* in bacteria-free culture was stimulated by anaerobiosis. He was also able to demonstrate an aerobic utilization of oxygen by the Warburg technique. It is difficult to interpret the finding of oxygen consumption in association with a stimulation of population under anaerobic conditions unless Riedmuller's classification of *Trichomonas foetus* as a facultative anaerobe is accepted.

Aerobic culture of *Trichomonas vaginalis* in this laboratory has always involved the incorporation of an infusion of beef liver in the culture medium. It has been demonstrated (unpublished data) that a reduction in the amount of liver infusion lowers the population level. In a study of carbohydrate utilization, Trussell and Johnson (1941) used a basic medium lacking liver infusion and found that it was necessary to establish anaerobic conditions by the addition of sodium thioglycollate before there was a measurable effect of carbohydrate on population. This stimulating effect of reduced oxygen tension is easily demonstrated by comparing the growth of stock cultures in an anaerobic jar with that in air.

In an attempt to throw further light on the anaerobic character of the organism a quantitative study of population at different oxygen tensions was undertaken.

MATERIALS AND METHODS

Experience gained in the early phases of this study led to alterations in the formula of the culture medium. In a fluid medium of low viscosity the population of trichomonads becomes much denser at the bottom of the culture. A more homogeneous culture medium and especially one in which the organisms will remain in suspension was prepared as follows:

Bacto liver infusion	80.0 ml[3]
Bacto heart infusion powder	25.6 gm
Bacto agar	0.4 gm
Distilled water	720.0 ml
Normal HCl	6.0 ml

This mixture was heated to boiling, tubed in 8.0 ml volumes and autoclaved. After cooling, 2.0 ml of sterile undiluted human serum adjusted

[3] Liver infusion was prepared by adding 75 gm Bacto dehydrated liver to 1 liter of distilled water, heating at 50° C for one hour and filtering after raising the temperature to 80° C.

to pH 6 were added to each tube. The pH of the final mixture ranged from 6.1 to 6.2. Choice of this medium was based upon preliminary investigations which revealed that a mixture containing 10% liver infusion and 20% serum plus the other ingredients listed would support a relatively dense population without significant shift in pH. Variations in population between cultures subjected to the same experimental conditions were also greatly reduced.

The inoculum was prepared by combining 3.0 ml of fluid from each of two stock cultures and adding 4.0 ml of the experimental medium without serum. This gave a suspension in which the organisms showed little tendency to settle either in the tube or in the pipette. The inoculations were made by transferring 0.2 ml of the fluid from this suspension of organisms to the mid level of each culture tube and stirring the culture thoroughly with the tip of the pipette.

In Series III the cultures were maintained under complete anaerobiosis, 100 mm oxygen tension and 500 mm oxygen tension. A fourth set of cultures was kept in an open rack as controls. Series IV was a duplication of Series III.

In Series V the culture conditions included complete anaerobiosis, 100 mm oxygen tension, pure oxygen, and a set of controls kept as before under atmospheric conditions. Series VI duplicated Series V.

Anaerobiosis was established in McIntosh and Fildes jars, improved model. The procedure followed was that recommended by the manufacturer. No evidence of impurity due to carbon dioxide was obtained when the hydrogen which was employed was allowed to bubble through filtered lime water for ten minutes.

The cultures were subjected to various oxygen tensions in McIntosh and Fildes jars by blowing a stream of hydrogen through the jar for five minutes, evacuating it to the desired negative pressure and then introducing oxygen to bring the manometer reading to zero. No evidence of carbon dioxide appeared when the oxygen which was employed was bubbled through filtered lime water for ten minutes. The highest concentration of oxygen was obtained by running a vigorous stream of the gas through an aluminum jar for five minutes. This jar was sealed by a gasket and the lid was tightened by adjusting three screw clamps at the edges of the lid. To what extent the resulting atmosphere was contaminated by residual nitrogen and other gases already in the jar and in the tubes is not known. The data from these cultures are labeled "barometric oxygen" subject to the above reservations.

Ten tubes were run at each oxygen tension, making a total of forty tubes for each series. At each counting period the four sets of cultures were removed from the incubator at the same time and brought to atmospheric conditions and room temperature by removing the lids from the

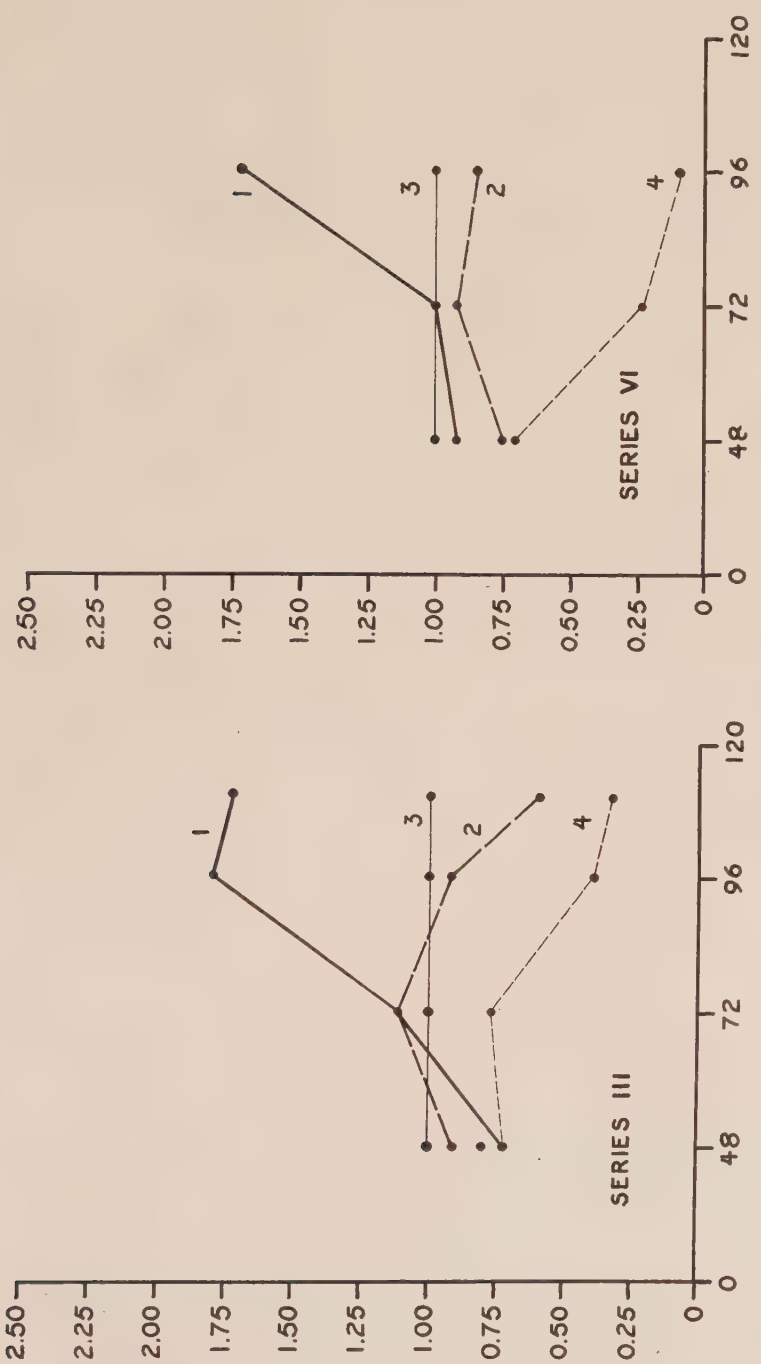

FIG. 2. Ratio experimental population over population in control cultures plotted against time. Curves as follows: Series III. 1—complete anaerobiosis, 2—100 mm oxygen tension, 3—controls, 4—500 mm oxygen tension. Series VI. Curves 1-3 as before, 4—barometric oxygen tension.

jars. It has been found that no multiplication of this bacteria-free culture occurs below 28° C (unpublished data). Therefore, it is assumed that the population values determined represent the result of multiplication while the cultures were subject to experimental atmospheres at the incubation temperature of 35° C. The latter temperature was adopted because the stock cultures had been maintained at this temperature for several months prior to the beginning of these experiments.

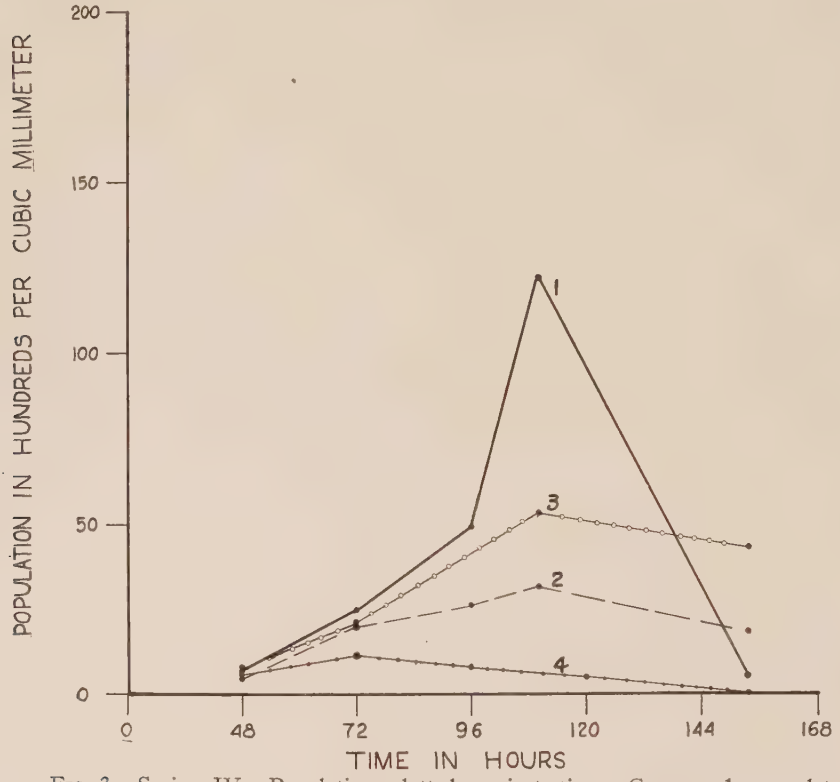

FIG. 3. Series IV. Population plotted against time. Curves: 1—complete anaerobiosis, 2—100 mm oxygen tension, 3—controls, 4—500 mm oxygen tension.

The pH values of the various cultures were determined with a glass electrode. In Series III and IV the reaction of tubes 1, 2, and 3 in each set was measured at the first three periods of observation. At the fourth and fifth periods it was determined for tubes 4, 5, and 6. A similar procedure was followed in Series V and VI except that it was unnecessary to make more than two successive determinations on any one culture since there were in all only four counting periods. To determine the error involved in diluting the culture fluid with two parts of distilled water, duplicate determinations on the diluted and undiluted culture fluid were made at the conclusion of Series III and IV. Populations were deter-

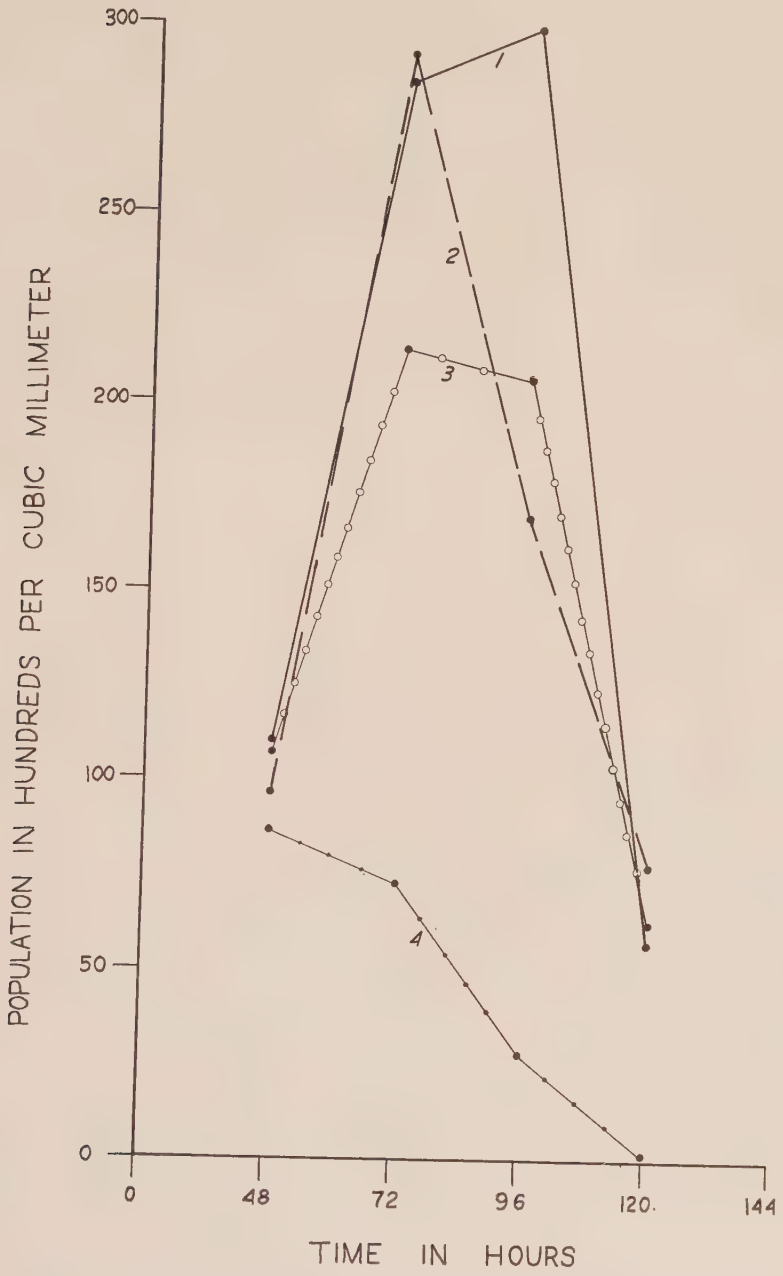

FIG. 4. Series VI. Population plotted against time. Curves: 1—complete anaerobiosis, 2—100 mm oxygen tension, 3—controls, 4—barometric oxygen tension.

mined with a hemacytometer. Following the first 48 hours of culture the measurements, unless otherwise indicated, were made at successive 24-hour intervals until a definite decline in population was observed.

Since the trichomonads produce only a faint clouding of this medium, gross contaminations were easily detected by direct observation. At the conclusion of each series all cultures were streaked on chocolate agar and the plates incubated aerobically in a moist jar for two days. A check for anaerobic contaminations was then made by keeping the same plates in a McIntosh and Fildes jar for two weeks. As a further check for anaerobic contaminations cultures 4, 5, and 6 in each set were inoculated into sodium thioglycollate medium containing glucose. No data are included from cultures shown to be contaminated. The contaminated cultures amounted to 4.5 per cent of the total and the data from these were excluded.

RESULTS

All tensions of oxygen employed in these experiments exerted some depression upon the population level. This fact is demonstrated by a comparison of the ratios of experimental populations to those of the atmospheric control cultures. In each case (Figs. 2, 3 and 4) the curve for cultures subjected to an atmosphere containing oxygen falls below that for cultures maintained under complete anaerobiosis. The depression of population by oxygen tensions of 150 mm Hg (controls) or less did not become significant until after 72 hours in any of the four series. The organisms did not appear to be sensitive to the difference of 50 mm oxygen tension between the control group (150 mm) and the cultures kept under 100 mm oxygen (curves 2 and 3, Figs. 3 and 4). No explanation is offered for the fact that in two out of four series the curve for 100 mm oxygen tension fell slightly below that of the controls. The depression of population in oxygen at barometric pressure was marked and continuous after the first 48 hours (curve 4, Fig. 4), whereas there was a slight rise in population under 500 mm oxygen between 48 and 72 hours (curve 4, Fig. 3). Complete anaerobiosis prolonged the period of population increase and constituted the optimum culture condition (curve 1, Figs. 3 and 4).

Very minor changes in pH occurred in the course of incubation and an average error of only 0.07 of one pH unit toward the alkaline side was introduced by diluting the culture fluid with two parts of distilled water.

DISCUSSION

Trichomonas vaginalis normally inhabits an environment in which it is probable that oxygen tension is low, particularly in the vaginal fornices. It is not therefore surprising to find that experimental populations rise to a maximum as oxygen tension approaches zero. Since it has been found

that this parasite is unable to utilize carbohydrates under aerobic conditions but can do so in the presence of a sulfhydryl compound like sodium thioglycollate (Trussell and Johnson, 1941), it seems probable that the –SH supplied by lived infusion may have contributed some measure of anaerobiosis in these experiments. Further investigation with a medium containing less liver infusion might reveal a more marked depression of population by oxygen. It was the purpose of this study to test the effect of oxygen tension when other conditions were optimum. The results make an investigation of the oxidation-reduction potentials in the culture fluid seem worthwhile.

SUMMARY

1. In these studies *Trichomonas vaginalis* has exhibited the characteristics of a facultative aerobe.

2. Oxygen exerts a marked depressing effect upon population.

3. Anaerobic conditions prolong the period of population increase and are optimum for the culture of the organism.

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CRENOSOMA ZEDERI N. SP. (NEMATODA: METASTRONGYLOIDEA), A NEW LUNGWORM FROM THE SKUNK (*MEPHITIS NIGRA*)

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During a recent investigation on the parasites of fur-bearers in New York State, this apparently new species of lungworm was found in the lower bronchi of two skunks, wild-trapped in Albany County. It is herein described and named in honor of J. G. H. Zeder, who described and named the first species of the genus *Crenosoma* in 1800.

Crenosoma zederi n. sp.
(Figs. 1–4)

Male: 1 specimen, 6 mm long, 330 micra maximum width, 100 micra wide just anterior to bursa. Cuticular folds present over body back as far as anterior ends of spicules. Folds 60 micra apart at base esophagus, increasing to 80 micra in middle of body, decreasing near end of folded region. Parallel longitudinal ridges over entire surface of cuticle. Esophagus 395 micra long, 58 micra in maximum width, near posterior end. Excretory pore opens 80 micra from anterior end. Bursa 200 micra wide, notched at ends of latero-ventral, postero-lateral and dorsal rays. Distinct ventral lobe present, notched at edge medioventrally. Rays stout. Ventrals divided at tips. Externo-laterals separate from medio- and postero-laterals which lie close to each other and to externo-dorsal, the last three rays being parallel and directed posteriorly at 45° angle from axis of worm. Dorsal stout and simple, 45 micra long and 28 micra wide. Spicules equal, lying close to each other, bent slightly ventrally at both ends. They are 265 micra long, 8 micra in maximum width, near anterior ends. Distal ends not distinctly swollen. Unpaired accessory piece 100 micra long, divided into coarsely granular anterior portion 40 micra long, half-moon shaped in lateral view, and smooth, narrow posterior portion 60 micra long, which tapers posteriorly and ends in sharp point.

Female: 5 specimens, measuring 24, 24, 25, 25, and 26 mm long, 480–560 micra in maximum width, 240 micra wide at base esophagus. Cuticular folds present over entire body; 80 micra apart at base esophagus, space increasing to 120 micra at vulva and decreasing to about 60 micra at anus. Longitudinal ridges as in male. Esophagus 480–560 micra long, 90–100 micra maximum width. Excretory pore 180–190 micra from anterior end. Vulva 10–10.5 mm from anterior end. Anus 160–170 micra from posterior end. Two lateral phasmids, not extending through surface of cuticle, 40–50 micra from tip of tail. Larvae in utero 17–20 micra in diameter, possess lateral alate swellings as described in *C. potos*.

Host: Mephitis nigra (Peale and Beauvois, 1796).

Locality: Delmar, Albany County, New York.

Type specimens: U. S. N. M. Helm. Coll. No. 45005.

The male of *Crenosoma zederi* can be distinguished from those of *C. striatum* and *C. taiga* by its lack of an obvious dorsal branch on the spicule. It differs from males of *C. vulpis* and *C. mephitidis* in the shape of the gubernaculum, stoutness of the bursal rays and in its lack of lateral

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projections at the tip of the dorsal ray. It differs from those of *C. potos* in the shape of the gubernaculum and stoutness of rays and can be distinguished from the males of all described species by the presence of cuticular folds over the body as far back as the anterior ends of the spicules. In describing *C. potos* Buckley (1930) mentions that the folds are represented by regularly spaced transverse smooth bands of cuticle on the posterior part of the males where the actual folding has ceased and that the longitudinal "ridges are present over the whole surface of the body, except immediately posterior to each fold, which is smooth for a short distance." These conditions are not observed in *C. zederi,* in which there are no interruptions of the longitudinal ridges whatsoever.

The females of *Crenosoma zederi* can be distinguished from those of *C. striatum* by the lack of a cuticular swelling at the vulva, and from all other species by the presence of cuticular folds over the entire body. Though the margins of the folds, as in other species of *Crenosoma,* appear to be spinous on superficial examination, with higher magnifications it is readily observed that this illusion results from the folding of the longitudinal ridges and that no actual spinous structure exists. Certain measurements and size relationships in both sexes of *C. zederi* differ from those of other species of the genus, but since there is some variability within each species and some overlapping in the measurements between the species, it seems advisable to minimize the quantitative and emphasize the qualitative characters.

In reviewing the characters of the described species of *Crenosoma,* I have noted some discrepancies on which comment seems desirable. Hobmaier (1941a) in describing *C. mephitidis* referred to Buckley's table of measurements of the known species of *Crenosoma* and remarked that *C. mephitidis* had certain resemblances to *C. vulpis.* In a later paper (1941b) he again emphasizes that *C. mephitidis* is morphologically similar to *C. vulpis* and that they might be confused. He (1941a) mentions as points of differentiation the presence of cervical glands in *C. mephitidis,* a size difference, especially in females, and the presence of a longer tail in females of *C. mephitidis.*

Buckley quotes part of Dujardin's (1845) description pointing to the presence of cervical glands in *C. vulpis* and Hobmaier himself (1941a) suggests that they may be a generic character. In view of the number of strongylins in which excretory glands have been reported it seems likely that their presence is a character common to a rather large group. At any rate they cannot be used to separate *C. mephitidis* from *C. vulpis.* Since worms of the same species may be expected to show differences in size when found in different hosts, it seems inadvisable to consider the difference between 15 mm length in females of *C. vulpis* and 18 mm in those of *C. mephitidis* a specific character.

Hobmaier's statement that the tail is longer in *C. mephitidis* is based on comparison of his measurements with those given by Buckley for *C. vulpis*. Buckley's table was compiled from measurements of Skrjabin and Petrow (1928) who gave, among other measurements for *C. vulpis*, the following: Accessory piece, 0.550 mm, tail, 0.870 mm. These measurements obviously are erroneous and undoubtedly represent typographical errors. Buckley gave these measurements in his table, following each with parenthetical question marks. He noted beneath the table that the figures queried probably should read "0.055 mm." and "0.087 mm.," apparently assuming that the error was simply in the position of the decimal point. No scale is given on the drawings of Skrjabin and Petrow, but checking the drawings against considerable material of *C. vulpis* in my collection and against measurements of organs given correctly in their text, it is evident that their drawings are correct and the correct measurement for the accessory piece is 125–135 micra and for the tail length 130–160 micra. Hobmaier gives the tail length of *C. mephitidis* as 140–150 micra.

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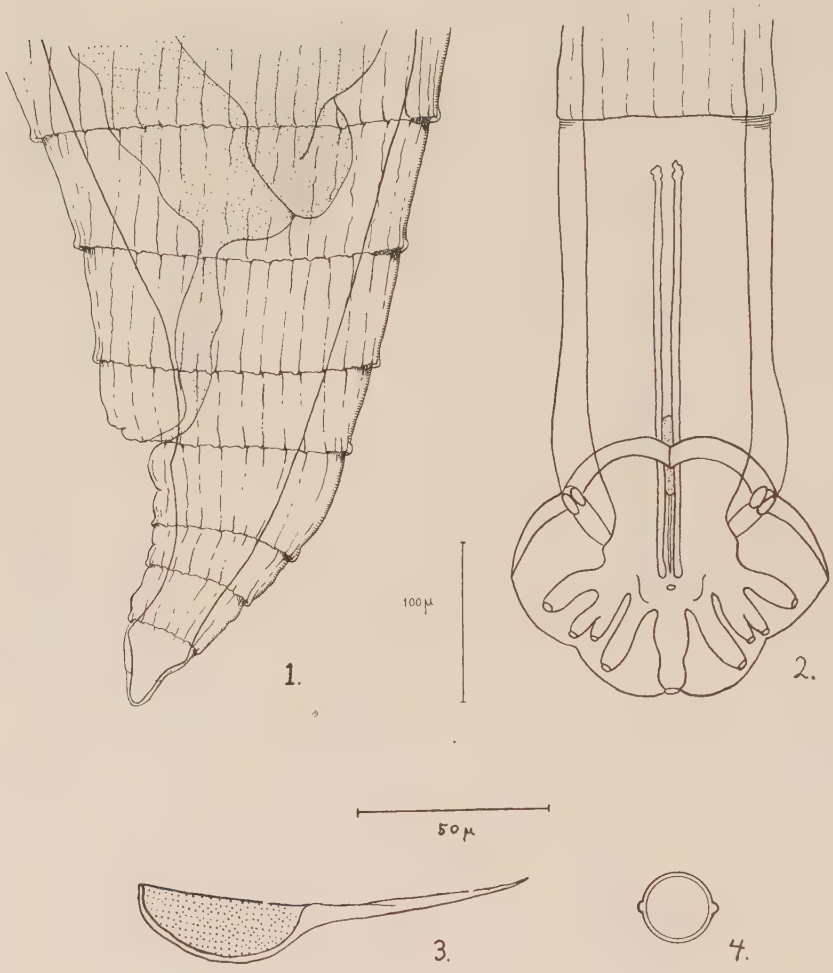

PLATE I

Crenosoma zederi n. sp.

FIG. 1. Posterior end of female, latero-ventral view.
FIG. 2. Posterior end of male, ventral view.
FIG. 3. Unpaired accessory structure, lateral view.
FIG. 4. First stage larvae, optical cross section.

(The vertical scale applies to Figs. 1 and 2, the horizontal scale to Figs. 3 and 4.)

A NEW SPECIES OF THE ACANTHOCEPHALAN GENUS *FILICOLLIS*

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In the late autumn of 1938 and the winter of 1940, I noticed dead and dying surf scoters (*Melanitta perspicillata*) and white-winged scoters (*M. deglandi*), all females, on the shore of Carmel Bay, Monterey Co., Calif. In 1938, a number of acanthocephalan worms were found in the intestines of a surf scoter and two white-winged scoters. On Feb. 24, 1940, I noted five dead surf scoters and one dead white-winged scoter. Beach scavengers had eaten the intestines of all but two surf scoters, which were parasitized by these acanthocephalans. The worms attached themselves by imbedding their hook-covered proboscides in the intestinal wall, their bodies hanging loose in the lumen (cf. Fig. A). In addition to these worms, four minute gizzard worms (nematodes), and one large tapeworm 250 mm posterior to the gizzard were found in the surf scoter taken Feb. 24.

All of the intestines observed were heavily infected. I examined in detail the alimentary tract of one surf scoter from the proventriculus posteriorly. Infection with the acanthocephalans began 250 mm posterior to the gizzard and continued to the anus, although the ceca were not infected. Worms became progressively smaller at the approach of the large intestine, although there were a few small worms scattered among the large ones throughout the small intestine. Infection was heaviest at the anterior end. According to my computations, there were 1,482 worms in the 28,000 sq mm of intestine.

The heavy infection of the scoters probably caused their death, or at least made them very susceptible to secondary infection. They had no subcutaneous layer of fat, and were too weak to cope with the waves or attempt to escape from approaching people or dogs.

Most of the worms mounted were fixed in hot 70% alcohol, stained in alum cochineal or Grenacher's borax-carmine, and occasionally counterstained with indulin blue dye. They were cleared in creosote-xylol, and mounted in balsam. Embryos were always counterstained in indulin blue dye, and were mounted in glycerin jelly.

On the basis of the slides examined, I propose a new species of the

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*I wish to acknowledge the assistance of Professor Harold Kirby (University of California, Berkeley), under whose guidance I carried on this research, and also that of Professor H. J. Van Cleave (University of Illinois, Urbana), who offered much helpful criticism on this paper.

genus *Filicollis,* which I name after Dr. Luther C. Altman, zoölogist, Lincoln High School, Seattle, Wash.

Filicollis altmani n. sp.

(Figs. 1–4; Text-Fig. A)

Body elongate, nearly cylindrical, with an even or uneven division of body surface by two strictures. Mature males 10.5 mm long (8.5–12); mature females 12.25 mm long (10.5–14). Diameter 1.67 mm (1.2–2.1). Neck long, slender, retractable,

TEXT-FIG. A. *Filicollis altmani* attached to the inner wall of the small intestine of a surf scoter. Photograph, natural size.

about 1.38 mm long (1.08–1.5); width at base 0.44 mm (0.3–0.55). Both sexes with minute body spines, confined to anterior part of body. Proboscis of both sexes characteristically oblately spheroidal, 0.7 mm × 0.8 mm (0.62–0.8 × 0.5–0.96); occasionally spherical or ovoid; bearing 28 (25–30) longitudinal rows of hooks with 11 (9–12) hooks per longitudinal row. Basal hooks 0.056 mm long (0.05–0.07); apical hooks, 0.038 mm long (0.03–0.05); and other hooks 0.047 mm long (0.03–0.06). Proboscis receptacle double walled, 2.2 mm long (1.7–2.75). Lemnisci about 0.05 mm shorter than the receptacle. Male genitalia occupy about two-thirds of the length of the body cavity. Testes spherical or ovoid. Four cement glands, long and tubular. Hard shelled embryos 0.065 × 0.026 mm (0.06–0.07 × 0.022–0.03); ex-

clusive of the shells, 0.041×0.012 mm (0.04–0.042×0.01–0.014). Embryos with three conspicuous shells; middle shell shape of ellipse with pointed ends, so showing slight tendency toward polar prolongation.

Type hosts: Melanitta perspicillata (Linnaeus) and *Melanitta deglandi* (Bonaparte) taken at Carmel Bay, Monterey Co., Calif. Nov. 11 and 18, and Dec. 4, 1938, and Feb. 24, 1940.

Habitat: Small and large intestine.

Holotype male (slide 3592.1 Van Cleave), allotype (slide 3592.2 Van Cleave), and paratypes at the Univ. of Ill. at Urbana. Paratypes at the Univ. of Calif. at Berkeley.

Filicollis altmani differs from *F. anatis* in the number of longitudinal rows of hooks on the proboscis, the number and shape of the cement glands, and the shape of the middle shell of the embryo. It differs from *F. sphaerocephalus* in the length of the lemnisci, number of cement glands, and the shape of the middle shell of the embryo.

EXPLANATION OF PLATE, p. 388

All figures drawn with the aid of a camera lucida.

FIG. 1. Holotype male of *Filicollis altmani.*
FIG. 2. Anterior end of paratype female.
FIG. 3. Middle section of proboscis of holotype male.
FIG. 4. Embryo from surface of mucosa of small intestine.

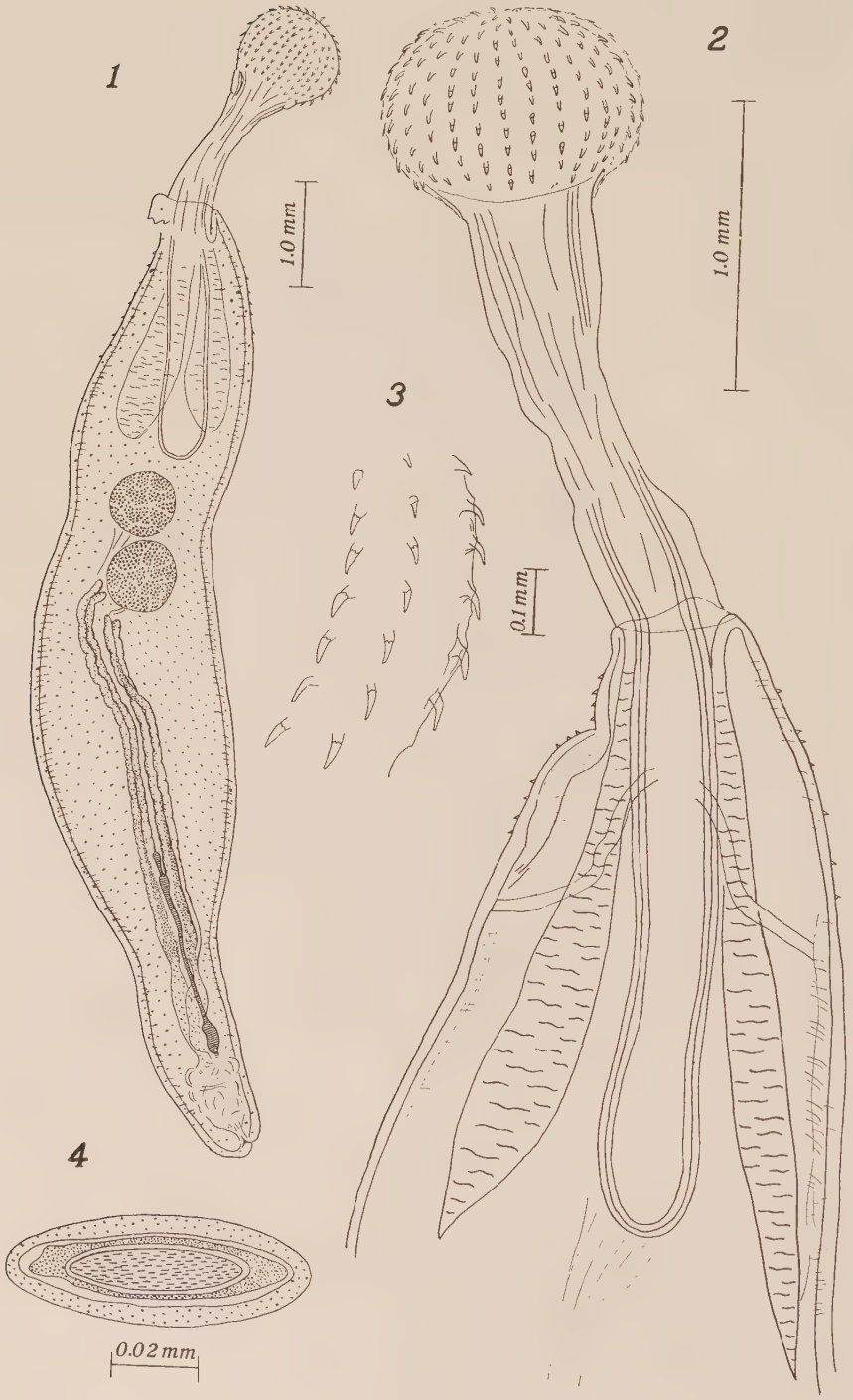

PLATE I

TRIGANODISTOMUM HYPENTELII N. SP. (TREMATODA: LISSORCHIIDAE) FROM THE HOG SUCKER, *HYPENTELIUM NIGRICANS* (LE SUEUR)[1]

JACOB H. FISCHTHAL

Examination of the small intestine of a hog sucker, *Hypentelium nigricans* (LeSueur)[2] collected on April 3, 1941, from the Saline River near Milan, Monroe County, Michigan, revealed three specimens of a new species of trematode belonging to the genus *Triganodistomum* Simer, 1929. In the fall of 1941, two of nine hog suckers taken from the Saline River near Saline, Washtenaw County, Michigan, contained three and four adult trematodes respectively. The following description is based upon the study of living specimens, and of whole mounts and transverse sections of specimens fixed in hot Bouin's fluid, stained in Mayer's paracarmine (whole mounts) or Ehrlich's acid haematoxylin (sections), and mounted in clarite X (Nevillite no. 1).

Triganodistomum hypentelii n. sp.
(Figs. 1–4)

Description: Triganodistomum. Body long, cylindrical, arched dorsally. Suckers and ventral surface spinose; margins of body spinose to anterior edge of posterior testis, with spines on ventral surface continuing postero-mesially from this level and terminating slightly beyond posterior testis; spines posterior to acetabulum arranged quincuncially; dorsal surface spineless, except dorsal to oral sucker. Oral sucker subterminal, smaller than acetabulum; latter prominent, protruding noticeably beyond ventral surface, at anterior fourth of body. Prepharynx short, narrow, muscular; pharynx globular, very muscular; esophagus slender, muscular; intestinal bifurcation at anterior margin of acetabulum; crura slender, dorsal to vitellaria, extending nearly to posterior end of body, left cecum usually longer than right, inner walls lined with single, conspicuous layer of cells. Vitelline follicles of varying size, 28–38 per side, mostly extracecal, posterior to acetabulum from anterior margin of seminal vesicle to middle of posterior testis. Paired vitelline ducts uniting ventral to ovary; common vitelline duct short, slightly swollen.

Ovary median, post-acetabular, trilobed, appearing as three separate structures; lobes smooth, approximately equal, uniting ventrally forming a small, slightly enlarged central portion at their junction; ovary filling intracecal space, slightly overlapping anterior testis dorsally. Oviduct short. Seminal receptacle small, arising from oviduct opposite origin of Laurer's canal about midway between ovary and oötype. Laurer's canal straight, heavy-walled, leading to opening on dorsum directly above anterior lobe of ovary. Mehlis' gland extensive, well-developed, of many cells. Uterus continuing slightly forward from oötype, recurving and descending to posterior part of body; coils fairly extensive, filling body as far forward as anterior margin of seminal vesicle; uterus ascending on left side of body; metraterm short, curved, muscular, thick-walled, postero-ventral to cirrus; genital atrium small. Ovary and testes arranged in series in posterior half of body.

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[1] Contribution from the Department of Zoology, University of Michigan.

[2] The names of fishes used in this report are those given in the keys of Hubbs and Lagler (1941).

Testes two, smooth, ellipsoidal, post-ovarian, approximately equal. Anterior testis slightly overlapping ovary ventrally, separated from posterior testis by uterine fold. Vasa efferentia dorsal to testes; right vas efferens leading from right antero-lateral margin of posterior testis, left from antero-medial margin of anterior testis; vas deferens short. Seminal vesicle internal, bipartite, innermost chamber about one-half as large as outer. Prostate gland cells numerous. Cirrus about two-fifths length of cirrus sac, inverted, protrusible, bearing long, slender spines at innermost portion, spines becoming abruptly shorter, more blunt toward genital pore. Cirrus sac long, curved, anterior portion slightly overlapping acetabulum dorsally, extending well posterior to latter. Genital pore ventral, almost on left margin, at level of middle of acetabulum.

Excretory pore terminal; bladder moderately thick-walled, long, tubular, bead-like in appearance, extending dorsal to testes as far as posterior fourth of ovary; primary collecting ducts forming loops dorso-lateral to oral sucker and receiving secondary collecting ducts posteriorly.

Eggs small, numerous, operculate, shell transparent; extruded freely from genital pore after placing worms in tap water; containing ciliated miracidium when deposited; no spontaneous movement visible within egg; latter not hatching in tap water. Miracidia squeezed from eggs showing presence of anterior papilla, primitive gut just posteriorly, and germ cells filling posterior half of body; cilia very long, longer than body of miracidium, arising in collar from anterior fourth of body and extending posteriorly; remainder of body devoid of cilia. Twenty eggs freshly extruded into tap water: 0.019 (0.019–0.020) × 0.013 (0.012–0.013) mm.

Measurements (in millimeters with the mean out of parenthesis and the minimum and maximum in parenthesis) of 3 adult worms mounted in toto: Body, length 2.155 (2.10–2.190), width 0.427 (0.405–0.465); oral sucker, 0.153 (0.150–0.158) × 0.160 (0.150–0.165); acetabulum, 0.215 (0.210–0.225) × 0.188 (0.180–0.195); prepharynx, length 0.028 (0.027–0.029); pharynx, 0.083 (0.078–0.091) × 0.095 (0.093–0.096); esophagus, length 0.120 (0.107–0.133); ovary, 0.228 (0.210–0.240) × 0.166 (0.155–0.179); metraterm, 0.149 (0.138–0.165) × 0.043 (0.040–0.045); anterior testis, 0.233 (0.210–0.250) × 0.148 (0.131–0.165); posterior testis, 0.266 (0.250–0.293) × 0.153 (0.142–0.165); cirrus sac, 0.350 (0.345–0.360) × 0.091 (0.088–0.093); seminal vesicle, 0.137 (0.131–0.144) × 0.081 (0.080–0.083); anterior end of body to anterior margin of acetabulum, 0.375 (0.330–0.405); posterior margin of acetabulum to posterior margin of cirrus sac, 0.262 (0.225–0.30); posterior margin of acetabulum to anterior margin of ovary, 0.380 (0.345–0.420); posterior margin of posterior testis to posterior end of body, 0.308 (0.30–0.315); ends of intestinal ceca to posterior end of body, 0.105 (0.096–0.115).

Host: Hypentelium nigricans (LeSueur).
Habitat: Small intestine.
Locality: Saline River, Monroe and Washtenaw Counties, Michigan.
Type: U. S. Nat. Mus. Helm. Coll. No. 44974; *paratypes* in author's collection.

T. hypentelii differs from all known species of *Triganodistomum* in having vitellaria whose anterior limits lie some distance behind the acetabulum instead of at the posterior half of it. The extension of the cirrus sac well posterior to the acetabulum differentiates it further from *T. attenuatum, T. mutabile* and *T. simeri.* It differs further from *T. mutabile* and *T. simeri* in having approximately three times as many vitelline follicles. It is further differentiated from *T. attenuatum* in possessing a deeply trilobed ovary, and from *T. translucens* in having the acetabulum at the level of the anterior fourth of the body.

The genus *Triganodistomum* was established by Simer (1929) from 15 specimens of *T. translucens,* the type species, from the small intestine

of a smallmouth buffalofish, *Ictiobus bubalus* (Raf.) from the lower Tallahatchie River, Mississippi. To this was added *T. attenuatum* and *T. simeri* by Mueller and Van Cleave (1932) from the intestines of common suckers, *Catostomus commersonnii* (Lac.) from Oneida Lake, New York; more information on infection with these two species was given in Van Cleave and Mueller (1934). The generic diagnosis was emended and further information was supplied to the descriptions of *T. attenuatum* and *T. simeri* by Mueller (1934). On further restudy of *T. attenuatum,* Mueller (personal communication) found the cirrus to be spined, although previously it had not been described as bearing spines; he suspects that in favorable material of *T. simeri* spines might be found on the cirrus also. Wallace (1939, 1941) demonstrated that *Cercariaeum mutabile* Cort, 1918 developed into an adult *Triganodistomum,* the new combination being *T. mutabile* (Cort); the adult worms were recovered from intestines of experimentally and naturally infected western lake chubsuckers, *Erimyzon sucetta kennerlii* (Girard), the latter from Waterloo Lake, Michigan.

The genus *Lissorchis* was established by Magath (1917) from specimens of *L. fairporti,* the type species, from the intestines of smallmouth buffalofish, *Ictiobus bubalus* (Raf.) and bigmouth buffalofish, *Megastomatobus cyprinella* (Valenciennes) from fish ponds at the Fairport Biological Station, Iowa. Wallace (1941) has drawn attention to similarities in the genera *Lissorchis* and *Triganodistomum,* and has placed the latter genus tentatively in the family LISSORCHIIDAE Poche, 1925, the validity of which must await further studies upon the related families, ALLOCREADIIDAE, PLAGIORCHIIDAE and MACRODEROIDIDAE.

KEY TO THE SPECIES OF THE GENUS *Triganodistomum*

1. Anterior limits of vitellaria posterior to acetabulum .... *T. hypentelii*
   Anterior limits of vitellaria at posterior half of acetabulum 2
2. 7–12 vitelline follicles per side 3
   16–32 vitelline follicles per side 4
3. Ovary deeply trilobed, appearing as three separate structures; length of body posterior to hind testis roughly equal to length of three gonads taken together ..... *T. simeri*
   Ovary moderately trilobed, not appearing as three separate structures; length of body posterior to hind testis roughly equal to one-fourth length of three gonads taken together ..... *T. mutabile*
4. Body long (3.5 mm); length posterior to hind testis roughly equal to length of both testes taken together ..... *T. attenuatum*
   Body short (1.0 mm); length posterior to hind testis roughly equal to one-third length of both testes taken together ..... *T. translucens*

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Explanation of Plate, p. 393

Fig. 1. *Triganodistomum hypentelii,* adult, ventral view.

Fig. 2. Diagram of female genital system, ventral view.

Fig. 3. Cirrus sac, showing bipartite seminal vesicle, prostate gland, inverted cirrus with spines, and genital pore; also muscular metraterm.

Fig. 4. Operculate egg containing miracidium.

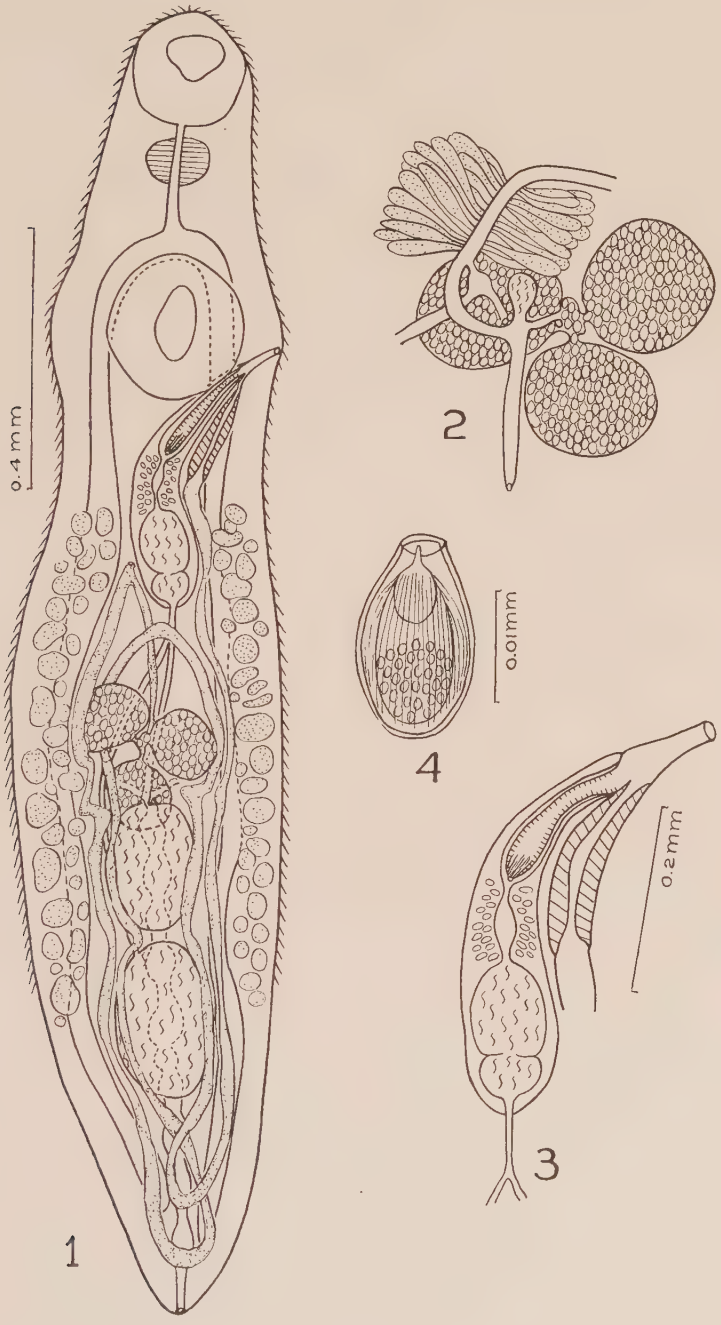

PLATE I

MECHANICAL TRANSMISSION OF RABBIT FIBROMA (SHOPE) BY CERTAIN HAEMATOPHAGOUS BUGS*

Cornelius B. Philip
Hamilton, Montana

The means by which Shope rabbit fibroma is maintained or transferred in nature is still speculative. The frequent location of the lesions about the legs and haunches in naturally infected animals suggests the possibility of some biting inhabitant of the nests or warrens acting as a vector. While there is no information of triatomid bugs infesting these locations in endemic areas in the eastern United States, the availability of laboratory colonies of certain species provided the opportunity to make simple mechanical transmission tests.

MATERIALS

It was the writer's good fortune to be located for a short period at the Johns Hopkins School of Hygiene where the facilities of the Department of Immunology were made available through the kindness of Dr. R. R. Hyde and other members of the staff to whom he is much indebted for various favors. The strains of rabbit fibroma virus used in the transmission tests, and of myxoma employed for immunity tests were those in routine use in that laboratory. The colonies of *Rhodnius prolixus* and *Triatoma infestans* were rearings from stocks obtained a few years ago during a visit of Dr. E. Dias of the Oswaldo Cruz Institute, Rio de Janeiro. The colony of *Triatoma protracta* was bred from specimens collected by Mr. Glen M. Kohls in Arizona in 1939. Domestic rabbits from a closely inbred colony were used as test animals.

EXPERIMENTAL DATA

An animal was given massive intradermal injections of virus on a clipped side and used as donor for subsequent bug feedings. A large, active fibroma developed rapidly. Nymphal and adult bugs were fed directly on the lesion on the fourth and seventh days. The first groups were labelled lots A1 and A2 and those fed on the seventh day were designated as lots B1 and B2. The A1 and B1 lots comprised insects used in alternate or interrupted feedings between the donor and a test animal at each period. The A2 and B2 lots represented bugs given initial complete feedings on the donor alone for later test after storage at

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* This study was conducted incidental to tenure of a Fellowship from The John Simon Guggenheim Memorial Foundation.

laboratory temperature. In three instances, additional animals were injected with saline suspensions of the viscera of insects used in prior feeding tests.

Protocols of transmission tests are given in Tables 1 and 2.

TABLE 1.—*Protocols of tests of mechanical transmission of fibroma virus by Triatomidae*

Lot	Species	Number of		Test animal	Myxoma challenge	Transmission results
		Bugs	Bites*			
A1 (4th day)	*T. infestans* *R. prolixus*	7 2	19 3	I	Survived (local lesion)	Positive; also transferred to following
	.....	.	..	Ia	Survived (no lesions)	Confirmatory transfer from I
	T. infestans	2	injec.	II	Survived (local lesion)	At least 1 or 2 of above 7 bugs contained virus
B1 (7th day)	*T. infestans* *T. protracta* *R. prolixus*	3 8 2	8 29 3	III	Survived (local lesion)	Positive
	T. infestans *T. protracta* *R. prolixus*	1 1 2	injec. " "	IV	Survived (scattered lesions)	At least part of previous bugs contained virus

* Certain individuals were induced to accept more than one feeding alternately on the donor and then on the test rabbit. Rabbits II and IV were injected with visceral contents of part of the bugs which bit rabbits I and III.

TABLE 2.—*Protocols of "incubation" test of fibroma transmission by stored bugs*

Lot	Species	No. biting	Interval*	Test animal	Myxoma challenge	Transmission results
A2 B2 B2	*T. infestans* *T. protracta* *R. prolixus*	6 7 1	15 12 12	V	Died	Negative
B2	*T. infestans* *T. protracta*	7 6	70 70	VI	Died	Negative
B2	*T. infestans* *T. protracta*	2† 4†	71 71	VII	Died	Negative

* In days after infected feeding.
† Gut contents injected.

Test rabbit I developed a small raised, inflammatory lesion at the site of insect bites beginning on the second day, attaining the size of a large garden pea on the third, and by the sixth had an erythematous area 15 mm in diameter. There being no further enlargement in the next three days, the fibroma was excised (ninth day), triturated in saline, and the extract injected intradermally into rabbit Ia. On the 45th day, no further evidence of infection having occurred, animal I was given an intradermal challenge dose of 10,000 units of myxoma virus, following which a lesion the size of a silver dollar developed at the site of injection with ultimate necrosis, but there was no further evidence of generalized infection. The animal was discarded on the 27th day after immunity test.

The transfer animal, Ia, developed a typical, though small fibroma at

the site of injection, and no reaction whatever occurred following a challenge test simultaneous with that of No. I.

Test animals II and IV developed small, subcutaneous nodules at sites of bug injection but no surface necrosis or inflammation of the skin occurred in either. On the 45th and 42nd days they received a challenge dose of myxoma virus simultaneously with rabbit I. No. II developed a local, ultimately necrotic lesion the size of a silver dollar at the site of injection and was discarded 27 days after immunity test. In No. IV, the infection became systemic with nodules on the ears, discharges from the eyes and nose, and respiratory difficulties; the animal was discarded on the 27th day, after survival and recovery were assured. It was apparent partial immunity also had been acquired by No. IV, though more feeble than in preceding animals.

Test animal No. III showed no lesions at the site of bug bites, but on immunity test on the 42nd day with myxoma, it developed a local necrotic lesion about the size of a half-dollar at the site of injection, and was discarded 27 days later. Since laboratory experience has indicated 100% fatal susceptibility of domestic rabbits to myxoma virus, it is evident that rabbits I to IV had been partially immunized by fibroma virus. (Rabbits V and a control died nine days after duplicate inoculums.)

Test animals V, VI, and VII showed no reactions following initial test, and died in 9, 10 and 10 days after immunity test, respectively. The myxoma inoculum of V duplicated the dosage given rabbits I to IV, while rabbits VI and VII also received 10,000 units of a later preparation of myxoma virus.

COMMENTS

It is well known (Shope, 1932; Shaffer, 1941; and others) that infection with fibroma virus immunizes against the invariably fatal myxoma virus in the domestic rabbit. Consequently, the latter can be used in immunity tests of fibroma-recovered rabbits.

It is presumed that mechanical transmissions by bugs given interrupted feedings on the donor (lots A1 and B1) were through the agency of contaminated mouth parts, by inference from experience in yellow fever studies with bedbugs (Philip, 1930) which are zoological relatives of triatomids and feed in a similar manner. In the yellow fever studies, 59 bugs of various stages did not transfer the virus during several interrupted, alternate feedings on an infected and a susceptible monkey, indicating that regurgitation of even minute amounts of ingested blood did not take place during feeding on the test animal since yellow fever virus has been shown to be infectious in extremely high dilutions.

Furthermore, it is believed in the present tests that the virus also was actually ingested, at least by the partially fed bugs used in the immediate transfer tests, so that quantitatively even more should have been imbibed

by the insects allowed to fully engorge on the donor rabbit without interruption, and stored for later "incubation" tests. The manner of feeding allowed only the inserted beaks of the bugs in contact with the fibroma, and the heads and appendages later were carefully snipped off previous to evisceration in the tests by injection so that the bugs tested in animals II and IV must have contained ingested virus to produce positive results. The negative results with incubated insects (Table 2) was not, therefore, due to failure to ingest virus during the original bloodmeals.

The tests, however, are obviously deficient in many respects for conclusions regarding insect transmission of fibroma virus. In addition to the use of unnatural insect hosts, the numbers of bugs in the two positive biting transfers was larger than would occur under natural conditions at any one time. Also the interruptions of other activities prevented the desirable determination of the actual duration of the virus in the bugs by an adequate number of intermediate tests. It seems not unlikely that some other species of biting insects could serve to transfer the virus at least in a similar mechanical manner. Whether arthropods of any species contribute to natural maintenance or transfer of fibroma virus remains to be demonstrated. If direct feeding on active fibromatous lesions is prerequisite to parasitic transfer of the virus, it is difficult to visualize the mechanism responsible for observed natural incidence of the disease. These experiments merely suggest one possible method of mechanical transfer.

SUMMARY

Two transmissions each of rabbit fibroma to domestic rabbits were obtained by interrupted feedings, and by injection of visceral contents of 3 species of triatomid bugs which had been fed immediately preceding on an animal infected 4 and 7 days previously. All 4 test rabbits survived immunity challenge tests with myxoma virus. Feeding tests of additional simultaneously, fully fed bugs were negative after periods of 12, 15 and 70 days storage at laboratory temperatures, as was also a test by injection on the 71st day.

It is considered probable that mechanical transfer was by the contaminated mouth-parts rather than by regurgitation, and that in addition the virus was actually ingested by the bugs.

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FURTHER OBSERVATIONS ON *PLISTOPHORA KUDOI*, A MICROSPORIDIAN OF THE COCKROACH*

VICTOR SPRAGUE AND JUANITA RAMSEY
Marshall College, Huntington, West Virginia

In the mid-gut of *Blatta orientalis* Linnaeus, collected from time to time on the campus of the University of Illinois, Urbana, Illinois, in the summers of 1937–1940, the senior author occasionally noticed protozoan spores, apparently of a microsporidian. This parasite may be identical with one of undetermined nature observed by Perrin (1905), although the spores he saw, being about 1–2 μ long by 0.5 μ wide on permanent preparations, were somewhat smaller than those described below in the living condition.

In the summer of 1941 the authors had access to the laboratory facilities of the Department of Zoology of the University of Kentucky, where a further study of the organism was made and its microsporidian nature confirmed. Since recent literature, including Kudo (1924) and Jírovec (1936), indicates no similar organism, it was described as a new species in a preliminary note (1941), and the name *Plistophora kudoi* proposed in honor of Professor R. R. Kudo.

MATERIAL AND METHODS

The host insects employed for the present study were collected on the campuses of Marshall College, Huntington, West Virginia, and the University of Kentucky, Lexington, Kentucky, in May and June, 1941, respectively. Pending examination they were kept in the laboratory in battery jars and fed a diet of apples.

The mid-gut and ceca of the host were extracted in 0.75 per cent NaCl solution. The organism was then studied in the living condition and later in stained smears or sections. The fixing solutions of Carnoy, Schaudinn, and Bouin were used with equally good results. The smears and sections were either stained with Heidenhain's iron-haematoxylin or treated by Feulgen's method. Good preparations were obtained by both methods; but the Feulgen reaction was not found particularly useful, since the nuclei of the parasite are so small that it is often hazardous to draw conclusions

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* Contribution from the zoological laboratories of the University of Kentucky, and Marshall College. The authors acknowledge their indebtedness to Dean William D. Funkhouser, Dr. Alfred Brauer, and Dr. Marvin C. Meyer for the courtesies extended in the use of the laboratory facilities of the University of Kentucky; and to Professor R. R. Kudo, of the University of Illinois, for authoritative advice during the course of the investigation.

regarding them. No attempt is made, therefore, to give a complete account of the life cycle in the present paper.

For demonstrating the polar filament, the structure was extruded mechanically and observed with dark field illumination according to the method of Kudo (1921).

OBSERVATIONS

Host-Parasite Relation

The incidence of infection is high in the localities mentioned above. Ten adult individuals collected in Huntington and examined a few weeks later were all infected. Twenty-nine of 42 adult individuals collected in Lexington and examined over a period of three weeks harbored the parasite. Thus, the microsporidian was observed in 75 per cent of all the host insects examined. This percentage applies about equally to males and females. Possibly some of the insects acquired the infection in the laboratory by close contact with those already infected. This incidence cannot, therefore, be regarded as truly indicative of the incidence in nature. Of 17 hosts examined in Lexington the next day after collection, however, 14 were infected. Thus, the incidence under natural conditions in this locality is approximately that given above.

A heavy infection can usually be detected by examining the ceca with the aid of a dissecting microscope. If the infected ceca happen to be distended with fluid, large opaque masses of spores are often seen in the epithelial lining. The masses tend to be arranged in longitudinal rows corresponding to the longitudinal plicae which occur in the epithelium, for the infection is the heaviest in the cells along the crests of these plicae. It is suggested that these particular cells are most heavily parasitized because their situation renders them most readily accessible to the invading organism. When a cecum contains a particularly heavy infection, great numbers of the epithelial cells are sloughed off and these, with the parasites, are seen as opaque masses lying free in the lumen.

The spores of the microsporidian are hardly discernible through the intestinal wall with a dissecting microscope. The gut contents, surrounded by a peritrophic membrane, can usually be removed very readily, however, and the masses of spores appear, with reflected light, as small white objects associated with the peritrophic membrane.

The relationship of the microsporidian to the host is one of true parasitism, for obvious damage is done to the epithelial tissue. Heavily infected cells are often greatly hypertrophied. The cytoplasm may become almost completely replaced with spores, and the distended cell membrane forms a sac which extends far out beyond the neighboring cells (Fig. 39) so as to resemble a budding process. The distal ends of these hypertrophied cells, packed with countless spores, often appear to break off and pass out into the lumen (Fig. 38). This process was noted with particu-

lar frequency in the ceca, where the lumina often contain enormous numbers of these remnants of epithelial cells packed with spores. Very frequently, however, the entire host cell is sloughed off, for a nucleus can often be seen within. Thus, large areas of epithelium, particularly that on the crests of the plicae in the ceca, become severely damaged.

Regarding the hypertrophy of the epithelial cells and the sloughing of parts of their substance, the phenomenon may be due largely to a mechanical process rather than to a specific tissue reaction. The hypertrophied cells typically contain such great numbers of spores that their bulk cannot be contained within the limits of a cell of normal size. Furthermore, cells which contain many parasites, but not in sufficient quantity to distend the cell bodies mechanically, are not noticeably hypertrophied (Fig. 40). Finally, the nuclei, which seem never to be parasitized, apparently show no specific reaction. Nuclei of infected cells and those of normal cells are the same in size and appearance (Figs. 39 and 40). In this respect the microsporidian differs from the majority of known forms. In sloughed off cells, of course, the nuclei undergo degeneration.

In summarizing the effect of the microsporidian on the host, it may be safely stated that considerable damage is done to the epithelial cells lining the ceca and mid-gut. No other effects, either microscopic or macroscopic, are evident. Whether the infection is ever fatal to the host is uncertain, for no data on this question are at hand.

The Spore

The mature spores in the fresh condition are about 3.2 μ long by 1.75 μ broad, highly refractive, reniform, pyriform or ovoid, and circular in cross section. At a relatively low magnification all the spores look essentially alike, but with high magnification much variation in size is seen (Figs. 1–7). Although the majority are approximately 3.0 μ in length, a few are as long as 5.0 μ. These long spores are scarcely any greater in diameter than the smaller ones, for this dimension does not seen to vary so much as the length. The typical shape is ovoid, though the anterior end is usually not plainly attenuated (Figs. 1 and 2). Upon close examination, however, many appear reniform (Figs. 5–7) and others pyriform (Figs. 3 and 4). In size and shape these spores greatly resemble those of *Thelohania reniformis* described by Kudo and Hetherington (1922). Distinct dimorphism does not seem to occur, for all intermediate conditions of size and shape seem to exist between the extremes. Moreover, much variation of spores in the same sporont is sometimes seen (Fig. 10).

Internally, the mature spores in the fresh condition appear absolutely structureless (Figs. 1–7). This is due, no doubt, to a relatively thick membrane, which also accounts for the great refractivity. Young spores, on the other hand, are much less refractive and contain a conspicuous

translucent area near one end resembling a vacuole (Figs. 8 and 9). This area is colorless in stained spores (Fig. 30).

The presence of a polar filament was easily demonstrated many times by Kudo's pressure method, as mentioned above. The filament is very delicate, uniform in diameter along the entire length, and relatively short (Fig. 29). Its length is approximately 25 μ in an apparently fully extruded condition. Rarely a filament as long as 50 μ was seen. Four to six undulations are often apparent in the filament, corresponding, no doubt, to the coiled condition existing before extrusion.

Developmental Stages

The stages preceding the schizont were not recognized. Presumably, the sporoplasm emerges from the spore membrane after ingestion by the host, makes its way to the specific site of infection, and eventually gives rise to the schizont stage. The authors believe the latter to be represented by certain elongated bodies (Figs. 11 and 37) which are very common in the cytoplasm of the epithelial cells. These objects are cylindrical, sausage-shaped, clavate, or fusiform and contain as many as ten small massive nuclei. The latter are usually arranged in a linear series and spaced at more or less equal intervals; sometimes, however, they are somewhat irregularly arranged. The infected cells may contain one to many schizonts. In the latter case, all the schizonts typically lie parallel in a compact group. They frequently lie also parallel with the long axis of the cell, due, perhaps, to mechanical causes.

Schizogony apparently results in the formation of small spherical bodies (Fig. 12), each with a single massive nucleus and with cytoplasm having somewhat less affinity for haematoxylin than the parent schizonts. Whether schizogony ever gives rise to multinucleate products was not determined. It is also not certain whether the schizogonic multiplication may be repeated, but it seems probable that it may. This inference is supported by the fact that cells containing many schizonts occur with great frequency. Repeated schizogony is a more plausible explanation of this frequency than multiple initial invasion.

The processes which initiate the sporogonic cycle are likewise not clear. Presumably, the young sporonts arise from the products of schizogony, but no distinguishing characteristics which might aid in identifying them were observed. Occasionally, small binucleate bodies as well as uninucleate ones were seen (Fig. 13). These may be young sporonts; they might also represent stages in schizogony or some other process. At any rate, whatever the initial stages may be, later stages are easily recognized. The young schizont develops by repeated nuclear division and simultaneous increase in the size of the protoplasmic body (Figs. 14–18). Frequently, a strand connects the two products of nuclear division

for a time (Figs. 17, 18, and 37). The cytoplasm of the developing sporonts has greater affinity for haematoxylin than that of the host cells or of other trophic stages of the parasite. Furthermore, the sporont, particularly if it is a larger one, characteristically lies in an apparent vacuole (Fig. 37) and is thus sharply set off from the host cytoplasm. This is not true of the other stages; thus they are studied with more difficulty because their boundaries are indistinct.

Eventually, the nuclear division ceases and the cytoplasm, which was formerly uniformly dark and homogeneous in appearance, begins to be differentiated into dark and light portions. The darker cytoplasm condenses around the nuclei, this being the beginning of sporoblast formation (Fig. 19). Later, the sporoblasts acquire their definitive ovoid shape and each, as seen in Heidenhain preparations, appears to have a single massive nucleus in the center of a colorless circular area near one end. These young sporoblasts are observed more readily in smears (Fig. 20) than in sections (Figs. 21 and 40), for in the latter they are usually closely packed together and are spherical in appearance. The residual protoplasm of the sporont, when it is apparent, is colorless and its limiting membrane distinct.

When observing the stages in the development of the sporoblast into a mature spore, one encounters great difficulties due to the extreme smallness of the objects. Apparently, as mentioned above, the young sporoblast is uninucleate at one stage. Older sporoblasts, as viewed in Heidenhain preparations, frequently appear to have more than one nucleus (Figs. 22–26). It was not possible to follow the stages in development, but it is probable that this process is accompanied by a complicated series of nuclear changes. Presumably, the various nuclear elements have different functions associated with the development of the various parts of the spore. In the mature spore a single dark-staining granule (nucleus?) is very frequently seen lying in the colorless area at the posterior end (Figs. 27 and 28). Except for the one area the mature spore typically stains very darkly (Fig. 30). In some cases, however, there is a small colorless area at the opposite end (Fig. 27).

The type of sporogony is that characteristic of the genus *Plistophora,* in which, according to Kudo (1939), the "sporont develops into variable number of (often more than 16) sporoblasts, each of which becomes a spore." Two to many spores develop in each sporont in the species under consideration (Figs. 31–36). The former number is rare, for only one of this type was observed. Those containing six, eight, ten, or twelve spores are common, but the majority contain sixteen or more. The size of the spores is not noticeably correlated with the number present within a sporont.

The mature sporonts are typically spherical and vary in diameter from

5 μ to 15 μ, the average being about 8 μ in the living condition. The diameter corresponds roughly to the number of sporoblasts or spores contained, but this correspondence is far from exact. When the number of spores is large, the interior of the sporont is usually completely filled and the spores lie closely applied to the limiting membrane (Figs. 35 and 36). When, on the other hand, the number is small, there is usually a rather wide zone of very hyaline and homogeneous protoplasm between the enclosed cluster of refractive spores and the distinct sporont membrane. Thus, because of the excess protoplasm in some sporonts, the diameter does not correspond closely to the number of spores within.

SUMMARY

Seventy-five per cent of 52 specimens of *Blatta orientalis,* collected at Huntington, West Virginia, and Lexington, Kentucky, contained *Plistophora kudoi* in the epithelial cells of the ceca and mid-gut.

The mature living spores are typically ovoid, often reniform or pyriform, very refractive, and show no internal structure. They measure about 3.2 μ long by 1.75 μ in diameter, but the size is highly variable. Distinct dimorphism does not seem to exist. A conspicuous colorless area is seen in the posterior end of stained spores. The polar filament is about 25 μ long.

The schizonts are elongate, being cylindrical, sausage-shaped, clavate, or fusiform. Schizogony results in several spherical uninucleate products, some of which give rise to sporonts.

Sporonts are subspherical when immature. At maturity they are spherical and contain a variable number of spores. Usually sixteen or more spores develop, this number being characteristic of the genus *Plistophora.* The membrane is often distinct and persistent until the enclosed spores reach maturity.

The only other known species of *Plistophora* in *Blatta orientalis* is *Plistophora* sp. Mercier, 1908, which has a different site of infection and much larger spores. These and other definite morphological differences justify the conclusion that the two species are distinct.

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EXPLANATION OF PLATE, p. 406

All figures were drawn with the aid of a camera lucida. Figs. 38 and 39 are magnified approximately 700 times and others 1500 times. Figs. 1–9, 29, and 31–36 are from living material; Figs. 10, 19–20, and 22 are from Schaudinn-Heidenhain smears; Figs. 11–18, 21, 23–28, 30, 37, and 40 are from Bouin-Heidenhain sections; Figs. 38 and 39, Schaudinn-Heidenhain sections.

FIGS. 1–7. Mature spores of various size and shape.
FIGS. 8–9. Young spores with "vacuole" in posterior end.
FIG. 10. Sporont in which the spores vary greatly in size and shape.
FIG. 11. A schizont.
FIG. 12. End products of schizogony.
FIG. 13. Binucleate stages of uncertain significance.
FIGS. 14–18. Stages in development of the sporont.
FIGS. 19–26. Stages in development of the sporoblast.
FIG. 27. A mature spore.
FIG. 28. A mature spore in end view.
FIG. 29. A spore with extruded polar filament.
FIG. 30. A mature sporont containing twelve spores.
FIGS. 31–36. Mature sporonts of various sizes, containing different numbers of spores.
FIG. 37. A host epithelial cell containing various stages of the parasite.
FIG. 38. A sloughed-off portion of an epithelial cell packed with spores.
FIG. 39. A number of epithelial cells from one of which a portion containing spores is being sloughed off.
FIG. 40. A cell packed with sporoblasts compared with normal ones to show that there is no change in the nucleus and little in the cytoplasm except in extreme cases.

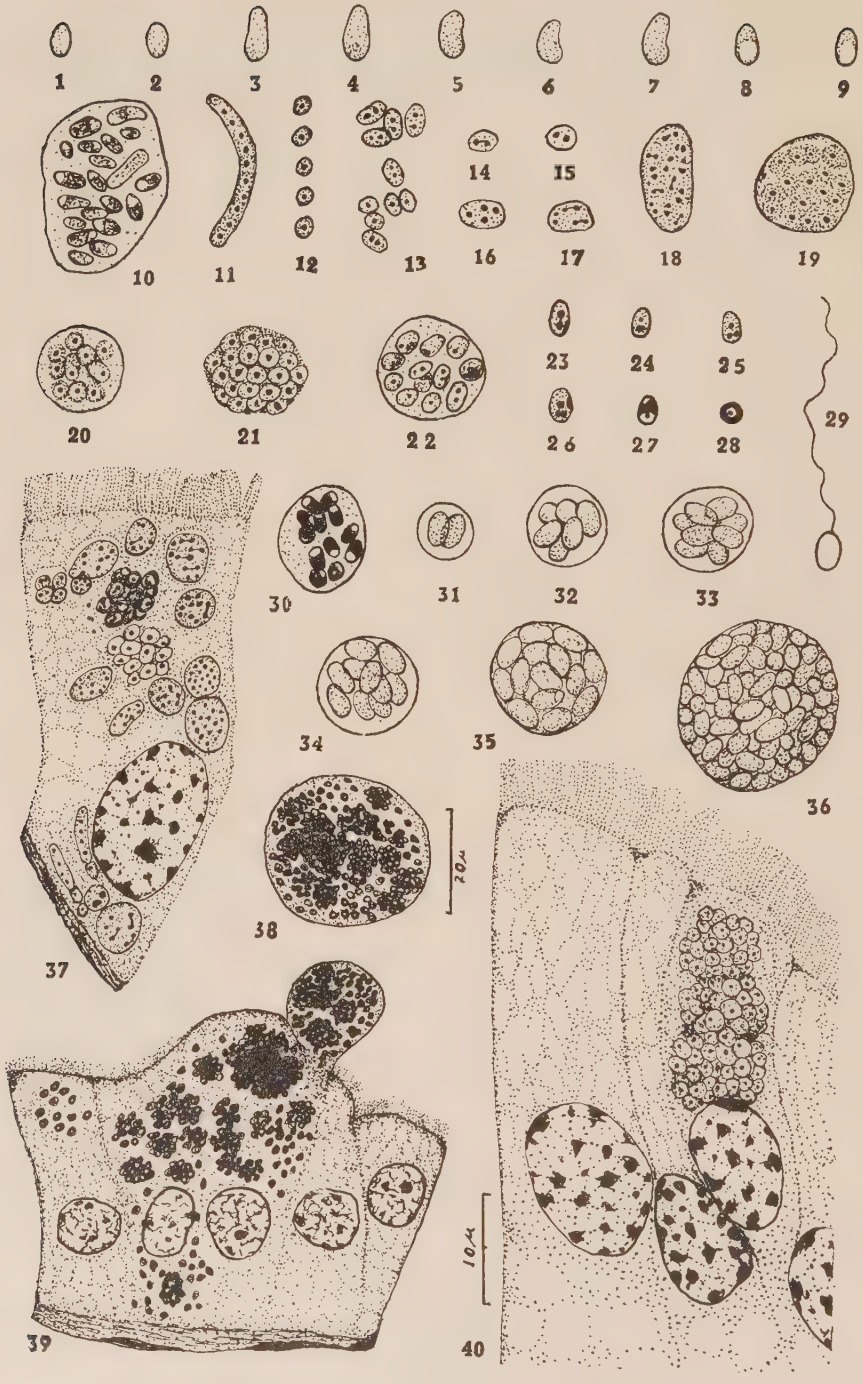
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PLATE I

STUDIES ON THE LIFE HISTORY OF *SIPHODERA VINALEDWARDSII* (LINTON) (TREMATODA: CRYPTOGONIMIDAE)

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During the summer of 1939, an undescribed pleurolophocercous cercaria of an unusual type was found in the small marine snail, *Bittium alternatum,* collected from Waquoit Bay, Cape Cod, Massachusetts. This cercaria has proved to be the larva of *Siphodera vinaledwardsii* (Linton, 1901), a common parasite of the toadfish, *Opsanus tau,* in the Woods Hole region. The cercariae encyst in flounders, killifish, and possibly other fishes which may be eaten by the toadfish. The present study throws considerable light on the affinities of *Siphodera* and related genera to other trematodes, a matter concerning which there has been some uncertainty.

S. vinaledwardsii was first described as *Monostomum vinaledwardsii* by Linton (1901) who observed the ventral sucker but interpreted it as a "genital acetabulum" and therefore a part of the copulatory apparatus. The species was subsequently recorded by Linton from hosts other than *Opsanus tau: Orthopristis chrysopterus* at Beaufort, North Carolina (1905); *Neomaenis synagris* and *Ocyurus chrysurus* at Bermuda (1907); and again from *O. chrysurus* at Dry Tortugas (1910). In the last paper, Linton proposed the genus *Siphodera* for the species, emended its description and erected the family Siphoderidae to include besides *Siphodera,* the new genera *Genelopa, Prodistomum, Stegopa* and *Metadena.* Manter (1934) expressed the opinion that these genera are not closely related and proposed the subfamily Siphoderinae, family Heterophyidae, to include *Siphodera* and a new genus, *Siphoderina.* Manter (1926) had previously redescribed *S. vinaledwardsii* from preserved material collected from toadfish at Woods Hole. In a revision of the superfamily Opisthorchioidea, Price (1940) has assigned the subfamily Siphoderinae to the family Cryptogonimidae. From serological studies, Wilhelmi (1940) has obtained evidence supporting the view that *Siphodera* is closely related to the heterophyids.

Some of the unusual characteristics of the cercaria of *S. vinaledwardsii* have been described recently for other opisthorchioid species. These include *Cercaria coronanda* Rothschild (1938) and the cercariae of *Centrocestus armatus* as described by Yamaguti (1938), *Caecinicola parvulus*

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by Lundahl (1939, 1941) and *Exorchis oviformis* by Komija and Tajimi (1940).

MATERIALS AND METHODS

Large numbers of snails were collected from seaweed near the shore of Waquoit Bay, near Menauhant, and isolated in finger bowls to obtain cercariae. Flounders were used almost exclusively to observe cercarial penetration and obtain metacercariae for study and experimental feeding; the larvae penetrated these fishes more readily than other species tried. Furthermore, the flounders would lie quietly with the fins well exposed so that penetration could be observed continuously with the microscope. In some cases, fishes were exposed only one day to cercariae so that all metacercariae were of the same age; others, particularly those to be fed to toadfish, were exposed to cercariae for several days to obtain massive infections. Flounders to be infected experimentally were first examined carefully with the dissecting microscope and any containing cysts or unusual pigment spots in the fins or skin were discarded. While a few cysts might have been present in the deeper tissues, it was established by dissecting a number of fish that if infected at all, there almost always were a few cysts in the fins. Considerable difficulty was experienced in keeping experimental flounders alive until the metacercariae were infective for the definitive host. Since it was impossible to obtain parasite-free toadfish, those used in experiments were isolated for at least one month so that experimental infections could be differentiated from natural infections by differences in the degree of maturity of the worms recovered.

Throughout the study, living material was used as much as possible although stained whole mounts of larval and adult stages and sectioned adults were used as supplementary material. All figures except those indicated as free-hand drawings are to scale and were made with the aid of a camera lucida. Measurements are in millimeters.

OBSERVATIONS

Experimental Proof of the Life Cycle

Before an attempt was made to determine the life cycle experimentally, metacercariae, believed to be those of *S. vinaledwardsii,* were found in the cardiac muscle of *Paralichthys dentatus.* These later proved to be identical with larvae found encysted in the fins and beneath the scales of the fish. The flame cell formula was the same as that of the cercaria but the excretory bladder was far more extensive, being Y-shaped, with arms reaching the oral sucker as in the adult *S. vinaledwardsii.* In experimentally infected fish, it was observed that the relatively small bladder of the cercaria developed in a few days to the extent observed in metacercariae from naturally infected flounders. Furthermore, the terminal genitalia-ventral sucker complex characteristic of *Siphodera* became well developed in metacercariae from experimentally infected fish.

In a preliminary experiment, a small flounder (*P. dentatus*) containing many 9-day metacercariae was fed to a toadfish which was killed and examined four days later but found to contain only a few mature worms from previous infections. Thus it seemed that 9-day metacercariae were not sufficiently developed to establish themselves in the definitive host. Another small flounder that had been exposed to cercariae continuously for seven days, was kept alive until the oldest metacercariae were 13 days old. This fish was cut into three pieces and fed to three toadfish, one receiving one-half of the fish, the others one-fourth each. The next day, one of the toadfish fed one-fourth of the flounder was killed and well over 100 very young *Siphodera* were recovered. Size variation of the worms corresponded with the differences in the ages of the metacercariae fed. In view of the previous experiment indicating that 9-day metacercariae were not infective, it seems very likely that well over one-half of the worms recovered would not have been present had the fishes been kept four days before examination.

Three days after feeding, the second toadfish, receiving one-fourth of the flounder, was killed and found to contain 30 small, immature *Siphodera* which were more uniform in size than in the first fish examined. Presumably, worms that had been encysted less than 10 days, failed to establish themselves. The third toadfish was killed 11 days after receiving one-half of the infected flounder. About 100 (95 were separated from the debris and fixed) young and 11 adult *Siphodera* were recovered. From none of three control toadfish were as many or nearly as young *Siphodera* recovered as from any of the experimental animals.

The morphological and experimental evidence, indicating that the life history of *S. vinaledwardsii* has been demonstrated conclusively, is supported by the fact that during an extensive survey of adult trematodes in the vicinity of infected snails we have not found any species whose larval stages might be confused with those of *Siphodera.*

DESCRIPTION OF STAGES IN THE LIFE HISTORY OF *S. vinaledwardsii*

The Adult

(Figs. 1–7)

The adult worms occur in large numbers throughout the intestine of the toadfish, *Opsanus tau.* Extended observations of both living and fixed specimens make it desirable to supplement the descriptions given by Linton (1901, 1910) and Manter (1926), particularly in respect to the excretory and reproductive systems.

The writers' specimens from naturally infected toadfish are somewhat smaller than those described by Linton and Manter but these differences are not significant since our specimens were not flattened. Six repre-

sentative mature adults, fixed in Bouin's fluid after shaking in saline, and stained with Mayer's paracarmine, have the following measurements: body length 0.93–1.19 (average 1.09), maximum width 0.48–0.65 (0.56); width of oral sucker 0.14–0.17 (0.156), pharynx 0.07–0.09 (0.08); diameter ventral sucker 0.07–0.09 (0.083), ventrogenital pit 0.08–0.11 (0.093), prostatic bulb 0.08–0.10 (0.085).

The body is thick and somewhat flattened, entirely covered with fine spines, and widest behind the middle. The anterior end is broadly rounded in fixed specimens but appears more pointed in living ones. The shape of the posterior end is characteristic (Fig. 1), always being decidedly pointed and occupied largely by the excretory vesicle. The median ventral surface of the hindbody bears a prominent longitudinal furrow, the depth of which depends on the degree to which the excretory vesicle is distended. There are numerous unicellular glands with ducts opening at the surface of the body. These are especially abundant in the forebody but a few may be scattered as far back as the opening of Laurer's canal. The granular contents of the glands stain intensely with eosin.

The small ventral sucker lies in a pit-like cavity, here called the ventrogenital pit, which opens to the ventral surface. This opening is variable in size and shape and the ventral sucker may be protruded through it. The genital pore is situated on the anterior wall of this cavity. The strong muscles radiating from the ventrogenital pit were described by Linton (1910) and Manter (1926) but these authors overlooked the conspicuous zone of gland cells surrounding the pit and apparently emptying into it. Also, in both living and stained specimens, the lining of the pit is distinctly cuticular in nature (Figs. 2–3), and is provided with conspicuous spines. These spines are continuous in distribution with those of the body surface and their points are directed towards the opening of the pit where they blend into the body spines (Fig. 3).

The excretory system (Fig. 7) is simple and can be observed readily in living, immature specimens under light coverglass pressure. The excretory vesicle is ventral in position, expanded, and Y-shaped. The stem is intercecal and widest posteriorly, narrowing somewhat anteriorly; the wall is irregularly crenulate. At the level of the ovary, the vesicle divides to form the voluminous arms which extend forward, intracecally at first, pass beneath the ceca at the esophageal level, and terminate as expanded sacs near the oral sucker. The convoluted main excretory tubules enter the arms of the bladder at a level just behind the ventral sucker, extend laterally a short distance, and divide to form anterior and posterior collecting tubules. Each collecting tubule receives two secondary tubules, each of which is joined by the capillaries of two flame cells, one dorsal and one ventral in position. The flame cell formula is ac-

cordingly $2[(2+2)+(2+2)]=16$ flame cells. The excretory pore is posterior and slightly ventral and connected with the vesicle by a slender canal.

Although there usually are four testes on each side, as many as six have been observed. They may be arranged one behind the other in the extracecal, dorsolateral regions of the hindbody, or one testis on either or both sides may lie median to the others and above the intestine. Beginning as an expanded, infundibuliform tube near each group of testes, a prominent vas efferens extends anteromediad, passing below the vitelline duct. The two vasa efferentia join a very short vas deferens which enters the seminal vesicle. The vesicle proper is divided into two parts of unequal length (Fig. 5), separated by a constriction. The terminal division is dorsal in position, elongate oval in shape, and provided with a thick wall, the outer layer of which is composed of cells which in optical section appear rectangular with slightly rounded outer edges. By focusing on the surface of the vesicle in living worms, these cells are seen to form a succession of transverse rings. Posteriorly, the heavy wall is constricted and continuous with the thin-walled posterior division of the seminal vesicle. This division is much longer than the anterior portion and sigmoid in shape; it extends ventrally in unflattened specimens (Fig. 1) but in flattened ones is usually deflected to the left and behind the terminal portion as shown in Fig. 5. The terminal anterior portion of the vesicle joins a prominent, spherical body, the prostatic bulb, which is situated above and slightly overlapping the anterior end of the ventral sucker. This bulb may be as large as the ventral sucker and is filled with pyriform glandular masses whose cytoplasm contains eosinophilic granules. These masses contain no nuclei and appear to be the inner ends of the large prostatic cells situated outside the bulb which is pierced by their ducts. A similar condition has been described for *Cryptogonimus, Caecinicola,* and *Allacanthochasmus* by Mueller (1934). On account of its structure and position in respect to the genitalia, the bulb is interpreted as an enlarged pars prostatica. From its anteroventral margin, a short ejaculatory duct extends abruptly ventrad to join the vagina. From this junction, a short genital atrium continues ventrad to the genital pore. There is no evidence of a gonotyl.

The ovary is situated midventrally, just behind the seminal vesicle. It consists of many lobes which obscure details of the female complex. The oviduct begins at the median dorsal surface of the ovary as an expanded, funnel-like tube provided with radiating muscles. This portion of the oviduct probably corresponds to the "swallowing apparatus" described by many authors, and functions in propelling the oocytes through the oviduct. The oviduct extends dorsally and anteriorly, passing below the vitelline receptacle, and is joined by the seminal receptacle and Laurer's canal. The receptacle is large, oval or pyriform in shape,

and situated to the left of the seminal vesicle. The junction of Laurer's canal with the seminal vesicle duct and oviduct forms a cross. From this point, Laurer's canal curves dorsally and then posteriorly above the vitelline receptacle, opening mid-dorsally above the ovary. From the cross, the oviduct turns sharply ventrad and is joined immediately by the duct of the vitelline reservoir. Just beyond this point, the oviduct turns posteriorly and joins the ootype in which the egg shell forms. An inconspicuous Mehlis gland is present. The beginning coils of the uterus extend posteriorly as a series of convolutions, and, in about one-half of the specimens, are confined to the right half of the body behind the ovarian level. Near the posterior end of the body, the uterus crosses to the left half of the body, passing beneath the excretory vesicle, and extends forward as a sinuous tube to the level of the seminal receptacle. It then recrosses the body to the right side and, in mature worms, coils several times in front of the right testes before extending anteriorly to the vagina. The course of the uterus as just described was observed in about one-half of the specimens studied, the remainder having either a mirror image of this pattern or an irregular one. Of 35 mature worms selected at random, 18 had the pattern described, 16 the mirror image, and one an irregular pattern. The eggs (Fig. 6) measure 0.02 to 0.022 mm by 0.011 mm in living worms and are colorless when first formed. As they pass through the uterus, the shells gradually become yellow and then brown, imparting a striking chocolate color to the pretesticular uterine coils in living worms.

The vitellaria are confined to the lateral regions and just beneath the dorsal surface of the body between the levels of the genital pore and the anterior testes. Medially, the transverse band of dorsal follicles does not reach as far anteriorly as the lateral fields, but its roughly convex posterior margin usually extends backward farther than the lateral follicles. A transverse vitelline duct extends from each lateral field to join the heart-shaped vitelline reservoir.

The Redia

(Fig. 8)

The redia is similar to that described for other pleurolophocercous forms, being elongate, without locomotor processes, and provided with a short intestine and birth pore near the pharynx. The excretory system of one side is shown in Fig. 8. The largest rediae observed were about 1 mm long and contained no mature cercariae. Hence it seems that the cercarial embryos leave the redia to complete their development in the snail's tissues as described for certain other pleurolophocercous forms.

The Cercaria

(Figs. 9–10)

Specific diagnosis: Marine pleurolophocercous cercaria. Body bell-shaped when strongly contracted; spinose as far back as eye-spots; general pigmentation lacking;

cuticle with fine "hairs." Measurements of living specimens: body extended over 0.23 long, contracted 0.10, moderately extended 0.17–0.19 (av. 0.18). Eye-spots block-like, 0.009 wide. Tail inserted ventrally, 0.24–0.40 long, averaging 0.29 when moderately extended; coiled when at rest; fin-fold continuous from near base of tail ventrally to posterior end of body dorsally, becoming very narrow for part of its extent on both dorsal and ventral sides of the tail. Oral sucker averages 0.03 by 0.025; pharynx 0.008 in diameter, situated just behind eye-spot level; remainder of digestive system undeveloped; ventral sucker 0.006 in diameter. 7 pairs of cephalic glands with ducts passing over oral sucker in two bundles; openings 3, 4, 4, 3 in crypt above mouth. Excretory vesicle U-shaped with irregular walls of thick, granular cells. Main excretory tubules short; anterior and posterior collecting tubules each receiving the capillaries of 2 pairs of flame cells; excretory formula $2[(2+2)+(2+2)]$. Genital primordium an undifferentiated mass of cells immediately anterior to excretory vesicle. Develop in simple rediae.

Host: Bittium alternatum (Say).

Locality: Waquoit Bay, Cape Cod, Massachusetts, U. S. A.

The cercaria of *S. vinaledwardsii* is unusual in a number of respects. Most noticeable are the insertion of the tail, the resting position of the larvae, and the presence of two instead of four bundles of cephalic gland ducts at the level of the oral sucker. In all described species of pleurolophocercous cercariae, the tail is set in a socket at the posterior end of the body, while in the present species, the tail is inserted in a slit-like opening on the ventral surface a considerable distance from the posterior end of the body. The posteroventral surface of the body is concave. The resting position (Fig. 10) is unique. In most if not all described pleurolophocercous species, the tail is sigmoid in lateral aspect when at rest and at the end of a swimming period, the larvae sink body downward through the water. In the present species, the tail curves dorsally, forming a spiral (Fig. 10) when at rest. If not disturbed, most of the larvae come to rest away from the light, either lying on the bottom or attached by the oral sucker to the bottom or side of the dish. A few rest on the side toward the light. There probably is a reversal of phototactic response with increasing age, since cercariae dissected from the snail are strongly photopositive. Swimming movements are similar to those of other pleurolophocercous larvae. If shadowed when at rest, the cercariae respond by swimming either toward or away from the light in a zig-zag path. If placed in a small container such as a Syracuse dish, they may swim back and forth across the dish one or more times before resting.

Various species of flounders, the killifish, *Fundulus heteroclitus,* and the stickleback, *Apeltes quadracus,* were tried as intermediate hosts by placing them in dishes with large numbers of cercariae. Flounders, especially *Paralichthys dentatus,* became heavily infected, the killifish obtained a few cysts, and the sticklebacks were free of metacercariae when examined. Small summer flounders were used for most of the experimental infections since the fins are transparent and the process of penetration could be observed continuously. If while swimming, the cercaria comes in contact with the fish and the oral sucker touches the epidermis

of the fin, the tail is lost instantly and floats away without further movements. After decaudation, there is a distinct cleft at the posterior end of the body. The secretion of the cephalic glands is immediately poured out around the oral sucker and the larva remains almost motionless for three to five minutes. It then begins to penetrate the epidermis slowly, with the posterior end motionless, the oral sucker and spines of the forebody apparently doing most of the work after the host's tissues are softened by the secretion of the cephalic glands. The larvae require 45 to 50 minutes to complete penetration, after which they migrate through the tissues, usually for only a short distance, and encyst within three hours. A small crater, with roughened edges persists for several hours at the site of penetration into the epidermis of the fish. In the fins, the larvae usually encyst near the fin rays although they may localize in the thin membrane between the rays. Many larvae encyst at the base of the fins and near the ventral surface of the body. They occur also in the deep tissues, including the myocardium.

Penetration is not influenced apparently by any chemotactic effect exerted beyond the epidermis of the fish since cercariae may swim against the fish or get beneath a fin and swim about for some time without penetrating. A definite contact between the epidermis of the fish and the oral sucker of the cercaria provides the stimulus necessary for penetration.

Metacercariae

(Fig. 11)

The young metacercaria is enclosed in a thin, oval cyst membrane of parasite origin. Dense, secondary cyst layers of host origin were not observed, nor was there any marked pigmentation around metacercariae two weeks of age. However, old cysts in the fins of naturally infected flounders were surrounded by a densely pigmented area. After encystment, the larva grows very rapidly for several days. The metacercaria at an age of less than one hour measures 0.115 by 0.09 mm; 24-hour metacercariae 0.145 by 0.12 mm; 2-day cysts 0.21 by 0.15 mm; 7-day cysts 0.27 by 0.18 mm; and six 9-day metacercariae averaged 0.28 by 0.19 mm. Shortly after encystment the body becomes opaque and filled with large vacuole-like cells (Fig. 11a). At the end of 48 hours, these cells are more numerous but more uniform in size (Fig. 11b). In 7-day metacercariae (Fig. 11c), such cells are still present but in smaller numbers and many features characteristic of the adult can be discerned. The intestinal ceca are developed, the arms of the excretory vesicle extend to the pharyngeal level and the characteristic ventrogenital pit complex is clearly seen. Although apparently in an advanced stage of development at 9 days of age, metacercariae must be at least 10 days old to be infective, as indicated by feeding experiments.

DISCUSSION

Although Witenberg (1929) clearly defined the gonotyl and ventrogenital cavity or pit, these terms have been misapplied to a considerable extent. The ventrogenital pit of *Siphodera* and related genera has been called also a gonotyl, genital sinus, genital atrium, genital sucker, pseudosucker and genital pit. Sections of *S. vinaledwardsii* clearly demonstrate not only that its cuticular lining is similar to and continuous with the cuticle of the body surface (Figs. 2–3) but also that it bears spines which differ only in size from those of the body. Cross sections of worms show clearly a continuous distribution and overlapping arrangement of the spines extending from the ventral sucker to the exterior body surface. The ventrogenital pit of *Siphodera* therefore is a secondary invagination of the body wall and not a genital atrium or gonotyl.

Certain genera of CRYPTOGONIMIDAE, as described by Mueller (1934), provide an interesting and significant series of modifications of the terminal genitalia-ventral sucker complex. Of these, *Caecinicola* seems to be the most primitive since it has neither a ventrogenital pit nor a gonotyl; in this genus, the relationship of the genital pore and ventral sucker to the body surface is essentially the same as in the ACANTHOSTOMIDAE, OPISTHORCHIIDAE and many other families of digenetic trematodes. The next in the series is the genus *Allacanthochasmus* in which a papilla-like gonotyl develops in front of the genital pore. In *Cryptogonimus,* the gonotyl is much more prominent, its base is pierced by the genital atrium, and the beginning of a ventrogenital pit is evident as an invagination of the body wall. The pit is well formed in *Siphoderina* and *Siphodera.* Since *Siphoderina* has a distinct gonotyl, its absence in *Siphodera* may be a secondary condition although the histological structure of the pit does not support that view.

In the HETEROPHYIDAE and CRYPTOGONIMIDAE, then, the tendency of the body to become invaginated in the region of the genital pore is expressed in varying degrees. The nature and contents of the resulting pit depend on the proximity and relationship of the genital pore to the ventral sucker. Thus in *Heterophyes,* the invagination forms a genital pit independent of the ventral sucker and enclosing only the sucker-like gonotyl, while in *Cryptocotyle* and many other genera, the invagination forms a ventrogenital pit, which, as its name implies, encloses both the gonotyl and ventral sucker. The genital pore and its associated gonotyl may be either anterior or posterior to the ventral sucker which becomes greatly reduced in certain genera, being merely a muscular thickening in the wall of the ventrogenital pit.

There also is an interesting series in the relationship of the genital pore to the gonotyl. Thus in *Acetodextra amiuri,* the genital pore is not closely associated with the gonotyl; in *Allacanthochasmus,* the pore is at

the base of the gonotyl; in *Cryptogonimus,* the genital atrium pierces the base of the gonotyl; while in other genera, the genital pore is at the extreme tip of the gonotyl. Since a true cirrus is lacking in these trematodes, the gonotyl probably functions as a cirrus or accessory copulatory structure, aided by the muscular ventrogenital cavity. Incidentally, there is a striking resemblance between the powerful muscles radiating from the ventrogenital pit in *Siphodera* and *Cryptocotyle.*

Knowledge of the life histories of *Exorchis oviformis, Caecinicola parvulus* and *Siphodera vinaledwardsii* indicate a close relationship between these and similar trematodes which Price (1940) has allocated to the family CRYPTOGONIMIDAE. In known life histories, the cercariae are all of the pleurolophocercous type with an excretory formula of $2[(2+2)+(2+2)]$. The cercaria described by Hopkins (1938) also is probably a larval cryptogonimid; although its excretory pattern was not determined, it agrees with the cercariae of *Caecinicola* and *Siphodera* in possessing seven pairs of cephalic glands and a bilobed ventral caudal fin.

Both *Cercaria coronanda* Rothschild, 1938, and the cercaria of *Centrocestus armatus,* as described by Yamaguti (1938), have an excretory formula of $2[(2+2)+(2+2)]$ but differ in other respects from the cryptogonimid cercariae mentioned above. *C. coronanda* has ten pairs of cephalic glands with ducts in four bundles and the ventral caudal fin is not bilobed, while in the cercaria of *C. armatus,* there are only four pairs of cephalic glands with two bundles of ducts figured and caudal fins are completely lacking. Disparity in respect to the extent of caudal fins and number of cephalic glands may be correlated with the degree to which a species depends upon swimming and active penetration in establishing itself in the second intermediate host. Ingles (1935) has described a pleurolophocercous species which encysts in the redia without escaping from the snail. In that species, a dorsal caudal fin is lacking and cephalic glands are neither figured nor described.

On the other hand, the identical excretory formulae in the cercarial stage of members of the families HETEROPHYIDAE, ACANTHOSTOMIDAE, and CRYPTOGONIMIDAE raises a question concerning the separation of these families as proposed by Price (1940). In our opinion, most if not all of the characters on which the separation is based are of doubtful validity. Certainly body shape and size and the presence of eye-spot remnants in adults depend on the nature and extent of post-cercarial growth; in large species, the eye-spot pigment, if present in the cercaria, may become completely absorbed or so finely dispersed that it is easily overlooked, while in small species, it usually remains distinct. In all three families, circumoral spines are present in some genera and absent in others. Gonotyls are present in members of the HETEROPHYIDAE, ap-

parently absent in the ACANTHOSTOMIDAE, and either present or absent in the CRYPTOGONIMIDAE. In other papers (Cable and Hunninen, 1940, 1942) we have expressed the view that modifications of the terminal genitalia are by themselves of doubtful significance as characters of certain families. The opecoelid genera, *Podocotyle* and *Opecoeloides,* provide a striking example in support of this interpretation. Their cercariae are so similar that they can be differentiated only with difficulty and yet *Podocotyle* has a prominent cirrus sac and no accessory sucker while *Opecoeloides* has no cirrus sac but possesses an accessory sucker between the ventral sucker and the genital pore. In the family CRYPTOGONIMIDAE alone, variations and gradations in the development of the terminal genitalia-ventral sucker complex indicate that this complex is not a reliable character for differentiating this family from the ACANTHOSTOMIDAE and HETEROPHYIDAE.

While there is an exact agreement in the known excretory patterns of cryptogonimids, members of the family HETEROPHYIDAE show considerable variation in this respect. The pattern in *Cryptocotyle* is complex but in the remaining known excretory patterns of heterophyids, there are four groups of two or three flame cells on each side of the body, two groups joining each anterior and posterior collecting tubule. If *Centrocestus,* with an excretory formula identical to that of the cryptogonimids, is properly allocated to the family HETEROPHYIDAE, the excretory pattern does not afford a valid basis for separating that family from the ACANTHOSTOMIDAE and CRYPTOGONIMIDAE. Price's (1940) subdivision of the OPISTHORCHIOIDEA is based chiefly on the shape of the excretory vesicle. In the HETEROPHYIDAE, the vesicle is triangular or Y-shaped with short arms not extending anterior to the ovary while in the ACANTHOSTOMIDAE and CRYPTOGONIMIDAE, the vesicle is V- or Y-shaped with arms reaching the pharyngeal level. It should be stated that in heterophyids with Y-shaped vesicles, the ascending tubules join the tips of the arms while in *Siphodera* and probably other cryptogonimids and the ACANTHOSTOMIDAE, the tubules enter the arms at a considerable distance from their anterior ends. Since there seems to be no distinctly intermediate forms, these differences may justify the separation of the family HETEROPHYIDAE from the ACANTHOSTOMIDAE and CRYPTOGONIMIDAE. It should be pointed out, however, that in the cercarial stage, there is little if any indication of the shape or extent to which the excretory vesicle will develop in the adult worms.

The excretory vesicle of *Siphodera* is distinctly Y-shaped with a long stem and thus agrees with that of the ACANTHOSTOMIDAE as defined by Price (1940). Furthermore, certain of the genera allocated to the CRYPTOGONIMIDAE agree with the ACANTHOSTOMIDAE in lacking a gonotyl, although the metacercaria of *Cercaria coronanda,* presumably an

acanthostomid, possesses a distinct gonotyl. The chief difference, then, between the ACANTHOSTOMIDAE and CRYPTOGONIMIDAE appears to be body shape. Although there may be significant differences in the arrangement of internal organs, many of these differences may be correlated with body size and shape. Therefore, it seems to us very doubtful that the ACANTHOSTOMIDAE and CRYPTOGONIMIDAE should be retained as distinct families. The adult of *C. coronanda* was not determined by Rothschild (1938) although she later (1940) expressed the view that it probably would be between the HETEROPHYIDAE (NEOCHASMINAE) and ACANTHOSTOMIDAE but closer to the latter. Since Price (1940) has placed the NEOCHASMINAE in the CRYPTOGONIMIDAE, it is quite likely that *C. coronanda* is a larval cryptogonimid. In view of that possibility, final disposition of the families ACANTHOSTOMIDAE and CRYPTOGONIMIDAE must await knowledge of acanthostomid life histories, none of which to our knowledge has been traced experimentally.

SUMMARY

The life history of *Siphodera vinaledwardsii* (Linton, 1901) has been traced experimentally and various stages in the cycle are described. The cercaria is a pleurolophocercous form developing in rediae in the marine snail, *Bittium alternatum.* Fishes, particularly *Paralichthys dentatus,* serve as the second intermediate host. Metacercariae in experimentally infected flounders were fed to three toadfish, *Opsanus tau,* all of which were found at autopsy to harbor young adults of *S. vinaledwardsii.*

In a discussion of the opisthorchioid trematodes, it is concluded that the separation of the ACANTHOSTOMIDAE and CRYPTOGONIMIDAE is of doubtful validity.

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Explanation of Plate I

All figures pertain to *Siphodera vinaledwardsii*.

Abbreviations

DG dermal glands
ED ejaculatory duct
EP excretory pore
ES esophagus
EV excretory vesicle
EY eye-spot remnants
GL glands around ventrogenital pit
GP genital pore
GS genital sinus or atrium
IN intestine
LC Laurer's canal
MG Mehlis' gland
MO mouth
MU muscle
OD oviduct
OS oral sucker
OT ootype
OV ovary
PG prostate glands
PH pharynx
PP prepharynx
PR prostatic bulb
SR seminal receptacle
SV_1 seminal vesicle, terminal portion
SV_2 seminal vesicle, beginning portion
UT uterus
VA vagina or metraterm
VD transverse vitelline duct
VG ventrogenital pit
VI vitellaria
VR vitelline reservoir
VS ventral sucker

Fig. 1. Adult, dorsal view.
Fig. 2. Adult, slightly oblique sagittal section.
Fig. 3. Adult, cross-section at level of ventral sucker.
Fig. 4. Free-hand drawing of spines in ventrogenital pit as observed in living adult worm.
Fig. 5. Free-hand drawing of reproductive system as observed in living adults under considerable coverglass pressure.
Fig. 6. Eggs.
Fig. 7. Adult, excretory system (free-hand).

Explanation of Plate II, p. 422

All figures pertain to *Siphodera vinaledwardsii*.

Abbreviations

CG cephalic glands
DF dorsal fin-fold of tail
EP excretory pore
EV excretory vesicle
EY eye-spot
GA genital primordium
GB germ balls
GD cephalic gland ducts
IN intestine
OG openings of cephalic glands
PH pharynx
VF ventral fin-fold of tail
VS ventral sucker

Fig. 8. Redia showing excretory system of one side.
Fig. 9. Cercaria, ventral view.
Fig. 10. Free-hand drawing showing resting position of cercaria.
Fig. 11. Metacercariae (a) 24 hours, (b) 48 hours, and (c) 7 days of age.

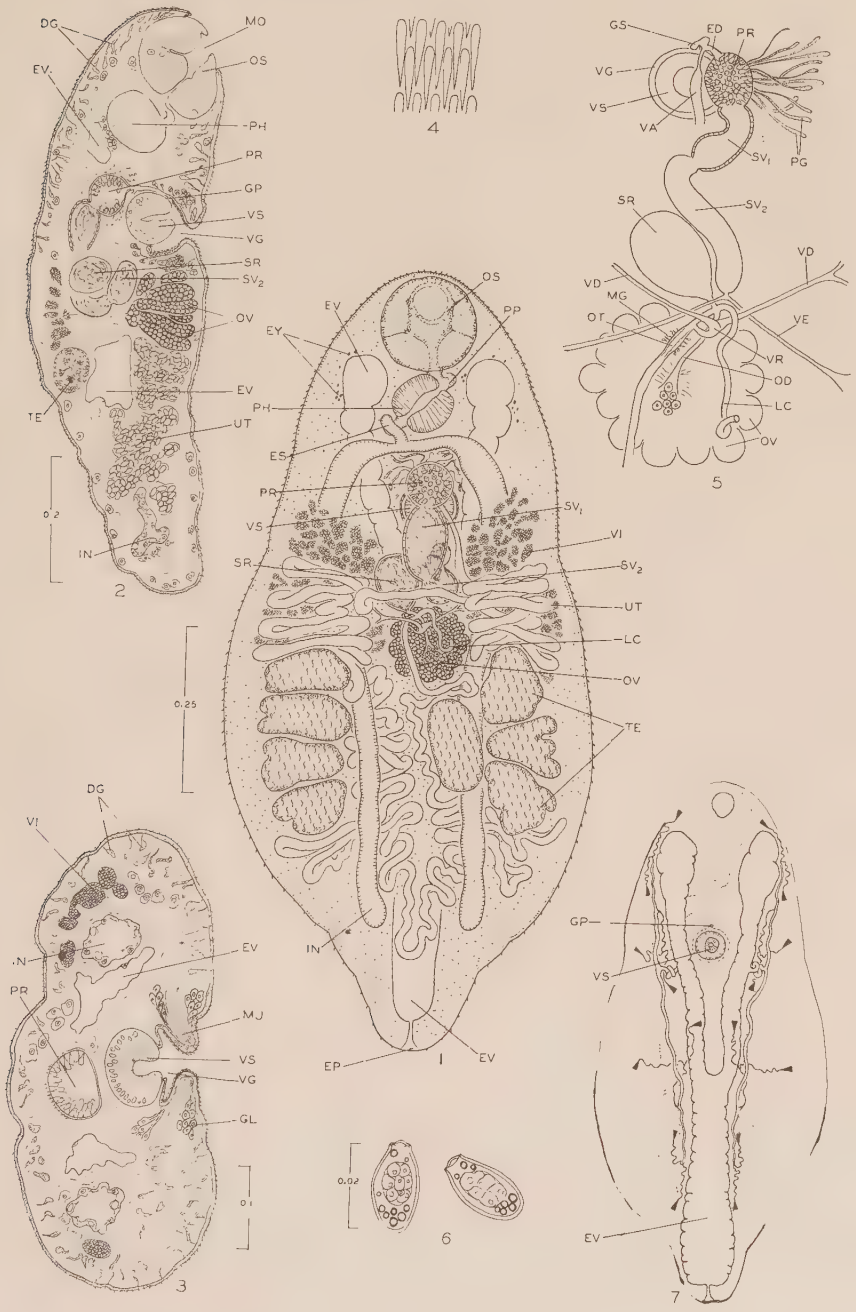
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PLATE I

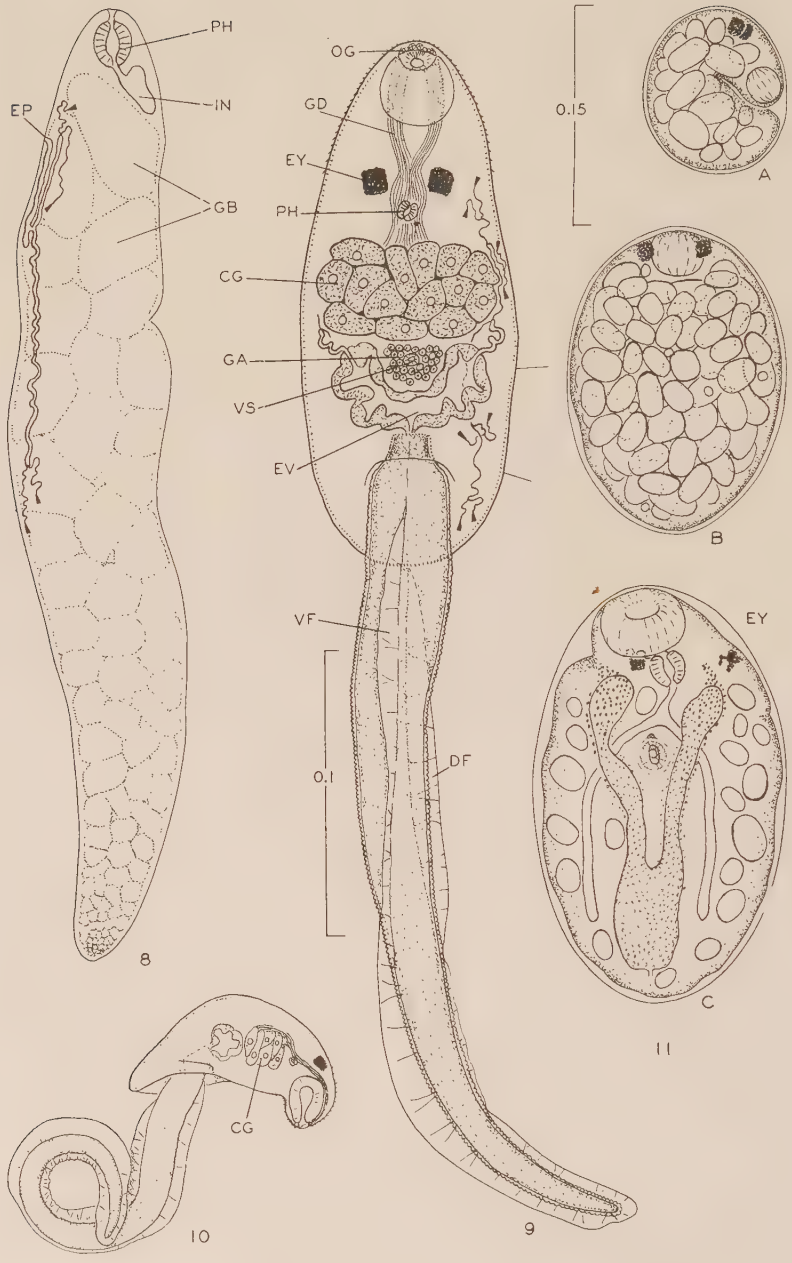

PLATE II

RESEARCH NOTES

PARASITES OF THE WOODLAND CARIBOU

Dikmans (1938, Proc. Helm. Soc. Washington 6: 97–101) had for his review of the parasites of semi-domesticated and wild ruminants no information on the helminths of the woodland caribou. Since that time, however, three of these hosts, *Rangifer caribou sylvestris,* have been examined for parasites in our laboratory at University Farm and one has been examined in the field in Canada and the helminths recovered from it submitted to us for identification.

Three of the four animals examined were captured by Mr. J. Manweiler at Montreal Lake, Saskatchewan in 1938. The first one, an adult female, was trapped on May 9, 1938. It died and was examined for parasites in the field at Montreal Lake by Mr. Manweiler. Several larval specimens of the tapeworm, *Taenia krabbei,* were removed from the muscles of its right shoulder, and 5 nematodes, *Dictyocaulus viviparus,* were taken from its lungs. It was also parasitized by 66 nose bots, *Cephenomyia phobifer,* 3 of which Mr. Manweiler reared. All of these parasites, with the exception of the 3 bots reared by Mr. Manweiler, were submitted to us for identification and have been placed in the helminthological collection of the Division of Entomology and Economic Zoology, University of Minnesota.

The second caribou, a month old calf, was captured on May 25, 1938. Only the viscera of the calf were available for study; no parasites were found.

The third animal, a yearling, was shipped from Montreal Lake, Saskatchewan, to the caribou refuge at Red Lake, Minnesota, where it lived for 3 years, dying on December 28, 1940. At autopsy 16 specimens of the filarial nematode, *Setaria cervi* [=*labiatopapillosa*] were found attached to the outside wall of its stomach.

The fourth caribou, an old female and one of the very few survivors of the native herd at Red Lake, Minnesota, which is the only region of the state where native caribou still survive, was found in a weakened condition by Mr. Manweiler about August 10, 1940. It was sent to University Farm for observation and then removed to the Carolos Avery Game Refuge in Anoka County where it died on August 18, 1940, probably due to the infirmities of advanced age. Three species of helminths were recovered from it: 15 lung worms, *Dictyocaulus viviparus,* 2 filarial worms, *Setaria cervi,* and 5 tapeworms, *Moniezia expansa.* In addition, Dr. R. Fenstermacher noted old liver abscesses, probably caused by the fluke *Fascioloides magna.* No flukes were found, but a few eggs were noted.

We are indebted to Mr. Allen McIntosh of the Zoological Division, Bureau of Animal Industry who made the specific identification of the tapeworms, and to Dr. G. Dikmans and Mr. John T. Lucker who verified our identifications of the nematodes. Also, we wish to thank Mr. Manweiler who spared no effort in getting the caribou to us.—Arnold B. Erickson, *Division of Economic Zoology, University of Minnesota,* and P. R. Highby, *Minnesota Conservation Department, Division of Game and Fish.*

A NOTE ON IMMUNITY REACTION IN THE BLACK-HEADED GULL (*LARUS RIDIBUNDUS* L.) INFECTED WITH *MARITREMA OÖCYSTA* LEBOUR, 1907

Very numerous American observers have drawn attention to the fact that: (1) very heavy infections of trematodes are not long supported by the host, which passes out numbers of mature and immature worms in the feces until the infection is greatly reduced; (2) reinfection is often unsuccessful, some type of immunity being set up.

During my work on the life-cycle of *Maritrema oöcysta* Lebour, 1907 a similar phenomenon was noted. One observation was made which seems worth recording.

Twenty thousand encysted cercariae were fed to a laboratory-reared black-

headed gull (*Larus ridibundus* L.) every day for eight days. The peak of the infection appeared to be reached about the sixth day, when vast numbers of viable eggs (which hatched on the third day), but no worms, were observed in the feces. From that date onwards the infection began to decline very rapidly, moribund worms in all stages of development being passed out, and the egg count fell precipitately. At the same time it became evident that large numbers of unexcysted worms were also appearing in the feces. At first it was thought that these were another closely allied species, accidentally fed to the bird, and were not developing in this particular host. This mistake can be made very easily, owing to the remarkable similarity between metacercariae of closely related species of *Maritrema*. I concluded, however, that they were in fact *M. oöcysta* cysts. Two possible explanations occurred to me. (1) Although the bird did not appear to be suffering from any intestinal disturbance due to the infection, as a result of the irritation caused by the worms, increased peristalsis passed the cysts so rapidly down the intestine that they avoided the action of localized enzymes normally responsible for the break-down of the cyst-wall. (2) That the production of the enzymes in question was greatly reduced or actually inhibited.

The cyst-wall of *M. oöcysta* is, however, remarkably susceptible to the action of enzymes, and artificial excystment can always be induced in the species with unusual ease. With these facts in mind ten days later I fed this gull several specimens of *Scrobicularia* sp., which I had experimentally infected with a few echinostome cercariae also obtained from *Peringia ulvae* Pennant, 1777. Ten days later several echinostome eggs, but no *Maritrema* eggs, were found in the feces. Forty thousand *M. oöcysta* cysts were again fed to the gull on three consecutive days. Subsequently fecal examinations revealed only an occasional *Maritrema* egg and a few echinostome eggs. Unfortunately enemy action terminated this experiment prematurely. It was intended to keep the bird alive for a considerable period, in order to discover how long an infection will last once it has become stabilized. This particular gull was killed by the effect of blast from a high explosive bomb, and the examination was consequently made only three months after the initial infection with *M. oöcysta*. When an infection of this worm is at its height, it is fairly well distributed along the entire length of the intestine, whereas in a stabilized infection the worms are confined to the terminal portion. In this particular bird only one worm was found in the upper end of the intestine, and the remaining specimens were all situated in the rectum.

It would therefore appear that in black-headed gull very heavy infection with *M. oöcysta* sets up an immunity reaction, which limits the number of the parasites, and to a certain degree prohibits the excystment of the metacercariae. On the other hand, this does not preclude reinfection with another species of trematode. —Miriam Rothschild, *Ashton Wold, Peterborough, England.*

ADELINA DERONIS N. SP., A NEW COCCIDIAN PARASITE OF THE AQUATIC OLIGOCHAETE, *DERO LIMOSA*

This parasite was discovered in the coelomic cavities of living specimens of the naid oligochaete, *Dero limosa*. The worms were from a small pond on the university campus. Examination of numerous specimens from 8 other naid genera collected in Pennsylvania, New Jersey and the New England states has not revealed infections of *Adelina deronis* or any other sporozoan. This apparent host-specificity justifies the name chosen for the new parasite.

Adelina deronis n. sp.

(Figs. 1–5)

Sporozoites: 12 microns long, 2.5 microns wide. Ovoid nucleus in posterior third.

Trophozoites (Schizonts): Spherical to ovoid. Mature stages 25 microns in diameter with up to 60 nuclei.

Merozoites: From 6 to 13 microns long, and 1.5 to 3 microns wide, depending on generation.

Microgametocytes: Sub-spherical, and from 7 to 9 microns in diameter. Each produces four flagellated microgametes, 2 microns long, 1.5 microns wide.

Macrogametocytes: Ovoid, averaging 19 microns in length, 17 microns in width.

Oöcysts: 17 to 21 microns in diameter, thin-walled and slightly lobed; 8 to 16 sporocysts.

Adelina deronis n. sp.

Fig. 1. Mature sporocyst containing two sporozoites and a central residual body.

Fig. 2. Advanced stage of gametocyte association. Pair is held together by copulation membrane. Nucleus of the (smaller) microgametocyte in early prophase. Conspicuous nucleolus and peripheral chromatin in macrogametocyte nucleus.

Fig. 3. Mature microgametocyte (drawn without the attached female) containing four flagellated microgametes and one nucleolar remnant.

Fig. 4. Zygote with attached microgametocyte remnant. Synkaryon spindle shows maternal chromatin (upper half) and paternal chromatin (lower half). Fertilization-membrane is slightly raised at lower pole, where microgamete has entered. Three unused microgametes left behind in the microgametocyte.

Fig. 5. Advanced oöcyst containing eight immature sporocysts. Microgametocyte remnant still attached at upper right.

Sporocysts: Thin-walled, spherical, 6 to 7 microns in diameter. Contain 2 sporozoites and a centrally located, granular residual body.

Host: Dero limosa.

Site of Infection: Peritoneal tissue and coelomic cavities.

Locality: Pond on campus of University of Pennsylvania.

Studies of living and fixed material, smears as well as sections, have shown the following life-cycle. Sporocysts (Fig. 1) are presumably ingested by the worm, and emerging sporozoites probably penetrate through the midgut wall into the coelom. Here they enter the cells of the parietal peritoneum, where they become enlarged, round up, and undergo schizogony. From 20 to over 60 merozoites are produced by each schizont. The vegetative cycle shows at least two morphologically distinct generations. It is possible to distinguish between vegetative and gametocyte-producing schizonts. The latter are of two types; one gives rise to an average of 30 microgametocytes, the other to an average of 8 macrogametocytes. These gametocytes become associated at an early stage of development. A syzygy membrane is formed around each pair (Fig. 2). After a period of growth, the microgametocyte gives rise to 4 flagellated microgametes (Fig. 3), one of which enters the female cell at a point opposite the region of original union of the gametocytes. Fertilization is followed by the secretion of a fertilization membrane. The maternal and paternal chromatin becomes arranged end to end in the synkaryon spindle (Fig. 4). This now undergoes condensation, and usually withdraws toward the pole nearest the microgametocyte remnant. The zygote nucleus is the only diploid nucleus in the entire cycle. The haploid chromosome number is established at the first zygotic division. The zygote (oöcyst), to which microgametocyte remnants are usually still attached (Fig. 5), produces 8 to 16 sporocysts, each with two sporozoites (cf. Fig. 1).

The mature oöcyst is the only stage suitable for species determination. *A. deronis* cysts are distinct from those of *A. octospora* Hesse (Sur le genre Adelea à propos d'une nouvelle coccidie des oligochètes. Arch. Zool. Exp., VII, XV, 1911), the only species with which they might be confused. The oöcysts of *A. octospora* are smoothly spherical, thick-walled, and 19 to 23 microns in diameter. They invariably produce 8 spherical, thick-walled sporocysts, 9 microns in diameter and containing 2 sporozoites each, in contrast to the characteristics given above for the new species.

A detailed cytological account of *A. deronis* is being prepared for later publication.—T. S. Hauschka and M. I. Pennypacker, *Zoological Laboratory, University of Pennsylvania.*

EXCYSTATION OF COCCIDIAL OÖCYSTS OF THE CHICKEN

In a paper by Pratt (1937, J. Parasitol. **23**: 426–427), it was reported that spores and sporozoites of the chicken coccidium, *Eimeria tenella,* were liberated in the crop of birds, in some cases as soon as five minutes after the oöcysts were ingested. His attempts to excyst the oöcysts in vitro with pancreatin, and with saline extracts of the crop, proventriculus, and duodenum failed. Although no positive statement was made, his results strongly inferred that digestive enzymes were not responsible for excystation. This is contrary to the findings of others, particularly Smetana (1933, Arch. Path. **15**: 175–192) who demonstrated, by well-controlled in vitro experiments, that trypsin was responsible for excystation of rabbit coccidia. This difference of opinion, in addition to the scarcity of definitive information prompted the writer to investigate the problem.

The pancreatic ducts were ligated in a number of coccidia-free White Leghorn chickens from five to twelve weeks of age. The ligated birds and normal controls were dosed into the crop with equal amounts of uniform suspensions of sporulated oöcysts. Fecal examinations for the resultant oöcyst discharge were made at suitable intervals by means of the sugar flotation technique. Negative results were checked further by the more sensitive test of feeding the incubated feces to coccidia-free birds. Ten gram samples of the feces in question were incubated for three days

at 30° C in shallow layers of 1½ per cent potassium dichromate solution. The incubated feces were then fed to coccidia-free birds and their feces, in turn, were examined for resultant infection in the usual manner. The objection might be raised that the intestinal epithelium of the ligated birds might be so altered physiologically that growth of the parasites could not be supported in any case. To answer this objection, infection of the ligated birds was attempted with merozoites (Levine, 1940, J. Parasitol. **26**: 337–343).

The results of these experiments (Table 1) were consistent. None of the birds with ligated pancreatic ducts became infected when dosed with sporulated oöcysts.

TABLE 1.—*Coccidial infection of normal chickens and chickens with ligated pancreatic ducts dosed with sporulated oöcysts and with merozoites. Infection determined by fecal examination for oöcysts (sugar flotation technique) and by feeding tests with incubated feces*

		Days after feeding sporulated oöcysts													
		4	5	6	7	8	9	10	11	12	13	15	17	19	27	29
E. praecox	Ligated	–	–	–									m3	+		
	Control	+	+	+												
E. mitis	Ligated		–	–	–	–		m3		+						
	Control		+	+	+											
E. maxima	Ligated			–	–	–	–		m4		+					
	Control			+	+	+										
E. hagani	Ligated				–	–	–									
	Control				+	+	+									
E. tenella	Ligated				–	–	–		–							
	Control				+	+	+								m5	+
E. necatrix	Ligated				–	–	–									
	Control				+	+	+				m5	+				

M3, m4, m5 = Merozoites (collected from a bird destroyed on the day of infection indicated by the number) injected into the intestine or into the ceca (*E. tenella* experiment).

This is apparent from the negative findings of fecal examinations. Feeding tests with the incubated feces were likewise negative. In the experiments with *E. tenella* and *E. necatrix* the control birds exhibited symptoms and post mortem lesions characteristic of infection with those species. The ligated birds, however, remained normal in every respect. On the other hand, in every instance where merozoites were administered, infection resulted promptly. This observation indicated that the intestinal epithelium of ligated birds was fully capable of supporting coccidial growth. Precautions had to be taken in ligating the pancreatic ducts, since imperfect ligation permitting even a small flow of pancreatic juice, rendered the bird susceptible to infection with sporulated oöcysts. In one instance where the ducts were not only ligated but also severed, slight infection resulted from a dose of sporulated oöcysts. This unexpected result was explained when the bird was examined post mortem. It was found that in addition to the three pancreatic ducts normally present in the chicken there was a fourth duct originating from the posterior end of the pancreas discharging secretions into the duodenum at the curve of the U. This anomaly had been overlooked when the other ducts had been ligated.

Since sporulated oöcysts had failed to infect ligated birds while merozoites were successful, it seemed logical to assume that excystation of the oöcysts had failed to take place. In that case the sporulated oöcysts should have passed through the intestinal tract unchanged. To test this hypothesis all the feces passed by the ligated and control birds in the experiment with *E. praecox* were collected during the 24 hours following the dosing with the sporulated oöcysts. The feces were stored in a refrigerator for 24 hours and then fed to each of two coccidia-free chickens. The relative number of sporulated oöcysts in each lot of feces was estimated by comparing the number of oöcysts discharged by these chickens. It was estimated that the

feces of the ligated bird produced 100 times as many oöcysts as those of the control. A similar experiment was done in the test with *E. tenella.* Again the chicken that received the feces of the ligated birds suffered an infection which was much more severe than the one occurring in the bird fed the feces of the control. This was evidenced by a comparison not only of the relative number of oöcysts discharged by each but also by the severity of the cecal lesions.

In the writer's opinion the only reasonable interpretation of the results herein described is that pancreatic juice is necessary for the excystation of oöcysts of the chicken. Some preliminary experiments in vitro done by the author indicate that trypsin may be the active agent as was found by Smetana with rabbit coccidia. Pratt's findings may possibly be explained either by assuming that a regurgitation of enzyme-containing fluid took place in the crop or that sporozoites were released in that organ as a result of mechanical pressure. In any event our data indicate that sporozoites, if liberated in that organ, could not produce infection.—P. P. Levine, *New York State Veterinary College, Ithaca, New York.*

A NEW THERMOSTABILE MEDIUM FOR THE PROLONGED BACTERIA-FREE CULTIVATION OF *TRICHOMONAS FOETUS*

A variety of media have been successfully employed for cultivation of bacteria-free cultures of *Trichomonas foetus,* including those of Glaser and Coria (1935, Am. J. Hyg. **22**: 221–226), Rees (1937, Am. J. Hyg. **26**: 283–291) and Daniel (1940, J. Parasitol. **26**: 85). It was found these would support growth sufficient to maintain cultures over an indefinite period of time, provided frequent transfers were made. Nevertheless, with the exception of Andrews and Lyford (1940, Am. J. Hyg. **31C**: 43–50) and recently of Lyford (1941, Am. J. Hyg. **33C**: 69–87), who were able to maintain individual cultures for as long as 3–4 months, none of the media developed support growth of individual cultures for long intervals (rarely longer than 3 weeks) and yet make subculturing possible.

The following notes are upon a thermostabile sodium citrate medium originated by Schneider (1941, Thesis, Univ. Wisconsin Library), and later modified by the addition of dried ground egg shell (1942, Schneider, J. Vet. Res., in press). A sufficient amount of this medium can be prepared in one day to last, when kept at ice-box temperature, for a number of weeks, with no resultant effect on the growth of *Trichomonas foetus.* It has supported the growth of two strains of *T. foetus,* one of which has been maintained on it for nearly two years, the other since its isolation on March 31, 1941.

At 37° C individual cultures, covered with "Parafilm" or in anaerobic glass-sealed test tubes (as used for transportation purposes), have lasted 30–60 days, with the longest on record 72 days. At room temperature, 22–25° C, such cultures have been kept 3–4 months. The longest positive cultures recorded were 139 and 147 days old, respectively. Subcultures were easily started from them. After prolonged storing at room temperature, cultures are placed in a 37° C incubator for 3 to 10 days to permit the flagellates to multiply in sufficient numbers (as noted by the development of gas bubbles at the surface of the liquid portion of the medium) to allow for making subinoculations. The medium is recommended for maintaining stock cultures of *T. foetus* at room temperature and is prepared in two parts.

A. Preparation of egg slants:

6 whole eggs

60 ml defibrinated bovine blood

50 ml of "Sodium Carbonate Solution" prepared as follows:

Sodium Chloride	6.5 gm
Sodium Bicarbonate	0.2 gm
Potassium Chloride	0.2 gm
Sodium Carbonate, Anhydrous, ($Na_2CO_3 + 10\ H_2O$ can be substituted)	0.5 gm

Potassium Phosphate, Monobasic (KH_2PO_4)	0.5 gm
Potassium Phosphate, Dibasic (K_2HPO_4)	0.5 gm
Glucose	2.5 gm
Distilled water	1000 ml

1. Beat up thoroughly with glass beads in a two-liter flask.
2. Filter through cheese cloth or gauze to remove membranes, etc.
3. Distribute in test tubes in 4 to 5 ml quantities.
4. Slant test tubes in previously heated autoclave, turn on steam till pressure reaches 10–12 pounds, and then turn steam off completely. Remove immediately when cool. It is to be noted that this does not sterilize the egg slants.

B. Preparation of liquid portion of the medium for covering the egg slants:

1. Sodium Citrate Solution

Sodium Chloride	5.0 gm
Magnesium Sulphate	0.2 gm
Ammonium Acid Phosphate ($NH_4H_2PO_4$)	1.0 gm
Potassium Phosphate, Dibasic (K_2HPO_4)	1.0 gm
Sodium Citrate	2.0 gm
Glucose	10.0 gm
Distilled water	1000 ml

2. Bovine serum (sterility not required) 5.0–7.5%. The serum is always centrifuged two to three times to remove all cellular material.
3. Adjust pH to 7.2–7.4.
4. Add 2 ml of a 1.6% alcoholic solution of bromcresolpurple (for visibly observing pH change, indicating flagellate growth in medium).
5. Add 40 mgm hematein (dissolved in distilled water so that each ml contains 5 mgm hematein. Ten minutes in the autoclave at 15 pounds pressure will help the hematein to go into solution).
6. Filter two to three times through double layer filter paper until the solution is *perfectly clear.*
7. Add dried ground egg shell on the bottom of the egg slant to a height of about 5–10 mm.
8. 10–12 ml of the filtered solution (direction #6) is used to cover the egg slant and dried egg shell.
9. Sterilize in autoclave 30 minutes, 15 pounds pressure.

Morris D. Schneider, *St. Paul, Minnesota.*

SOUTH AMERICAN CUTANEOUS LEISHMANIASIS IN EXPERIMENTAL ANIMALS

Under the name leishmaniasis are included the human diseases caused by protozoa belonging to the genus *Leishmania.* The clinical forms of the disease may be classified under at least two groups, visceral and cutaneous. In the cutaneous disease, which occurs in South and Central America, India, Asia and Africa, single or multiple ulcerating granulomatous lesions are found on exposed parts of the body, and no invasion of the viscera is reported. In the American form of cutaneous leishmaniasis, the mucous membranes of the nasopharynx may also be affected in 10 to 50 per cent of cases in particular localities.

The etiological agent of South American cutaneous leishmaniasis, named *Leishmania brasiliensis* Vianna (1911, Brazil-Med. **25**: 411), may be recovered from the skin lesions in smears and cultures under favorable conditions. Isolation is frequently made difficult by reason of secondary bacterial infection of many lesions.

Experimental attempts to produce the disease in many different animals have been attended with varying degrees of success (Geiman, 1940, J. Parasitol. **26** Suppl.: *22–23*). A fundamental problem has been to find an animal that is uniformly susceptible to cutaneous infection, thereby enabling one to carry out critical studies

of transmission, immunology, and comparative pathology of the disease. In the present investigation animals have been used which are closely related to species known to be susceptible to visceral leishmaniasis. Thus we have studied Syrian hamsters, *Cricetus auratus,* Texas ground squirrels, *Citellus tridecemlineatus* subspecies, and a few of the members of the Sciuridae indigenous to the eastern United States.

The organisms used for inoculation of animals were isolated in 1939 in Peru from human cases of cutaneous leishmaniasis (Geiman, 1940). They have been maintained subsequently through serial passage on the well-known NNN medium of Novy, MacNeal and Nicolle (Nicolle, 1908, Bull. Soc. Path. Exot. 1: 121–126). Material obtained from such cultures, suspended in normal saline, has served for inoculation of animals by subcutaneous or intraperitoneal routes, or combinations of both. After inoculation, an animal is observed at regular intervals for signs of disease. If a cutaneous lesion appears, it is punctured under aseptic precautions to demonstrate and recover organisms. Some of the material thus obtained is planted on NNN medium, and the culture is examined for flagellates until it is a month old. Smears of material obtained by puncture are fixed in absolute methyl alcohol, stained with Giemsa solution, and examined for cells containing parasites in the form of typical Leishman-Donovan bodies. An animal is declared to be positive only after it has developed a gross lesion from which characteristic forms of the parasite have been recovered in culture or in smears. Biopsy specimens have been taken from certain lesions from time to time for implantation into other animals and for sectioning. When an animal is autopsied, routine smears and cultures are made of heart's blood, spleen, liver, and bone marrow.

This study of three strains of *Leishmania brasiliensis* has given the following results:

Syrian hamsters have shown no evidence of infection following intraperitoneal injection of culture material. Fourteen animals have been injected subcutaneously. Only three of these have developed skin lesions, and they were inoculated with organisms which had been subjected to passage through chick embryos (Geiman, 1940). The lesions consisted of indurated nodules which were first noticed at the injection sites 55, 74, and 90 days after inoculation. The nodules gradually increased in size to a diameter of 5–7 mm. The overlying skin has remained intact, and the lesions have failed to ulcerate. The lesions have persisted in two of these animals for eleven months, at which time the parasites could still be recovered in smears and cultures. There has been no apparent effect on the general well-being of the animals. The third positive animal died of unknown cause eight months after inoculation, and, owing to an accident, no autopsy was performed.

Ten Texas ground squirrels have received series of four intraperitoneal injections of culture material at short intervals. No evidence of infection has been found in these ten animals during life or at autopsy. Five of these squirrels, subsequent to their series of intraperitoneal injections, received subcutaneous injections of the same strain with which they had been injected intraperitoneally, but no lesions developed. A sixth squirrel, however, was injected subcutaneously with a strain different from that used for intraperitoneal injection. A nodular cutaneous lesion was first noticed 97 days later at the site of injection. This has persisted as a small nodule during the ensuing eight months, with no signs of ulceration or regression, and organisms have been recovered at intervals.

Subcutaneous injections of culture material were given to eight normal Texas ground squirrels, and all eight animals developed nodular skin lesions after periods ranging from 21 to 44 days. In every case the organisms have been recovered in smears and cultures. The nodules have subsequently ulcerated, and have persisted in the latter form for more than 11 months in some cases. Serial passage to other ground squirrels and to hamsters has been effected, with production of cutaneous lesions. Thus Koch's postulates have been fulfilled.

A red squirrel, *Sciurus hudsonicus loquax,* was injected subcutaneously with culture material, but this animal died of unknown cause two weeks later.

Two gray squirrels, *Sciurus carolinensis leucotis,* and one chipmunk, *Tamias striatus lysteri,* have developed nodular skin lesions following subcutaneous injection. The two squirrels were killed and autopsied soon after the nodules had begun to develop. The lesion of the chipmunk regressed after three months without undergoing ulceration. The organisms were recovered from each animal.

The pathology of experimental South American cutaneous leishmaniasis in squirrels has been studied. It is similar to the reaction produced in human beings, consisting of an accumulation of mononuclear phagocytes containing the parasites. An indurated papule is formed after an incubation period of about three to eight weeks. This increases in size and may ulcerate. A notable difference, however, lies in the observation that the ulcerated lesions of the squirrels remain remarkably free from evidence of secondary bacterial invasion, which is quite common in the human skin lesions. Furthermore, we have not observed invasion of the nasopharynx in squirrels.

The results of the present study suggest the usefulness of ground squirrels in the investigation of South American cutaneous leishmaniasis. Further work is in progress at the present time, and special attention is being directed toward the pathology and immunology of the disease.—H. S. Fuller and Q. M. Geiman, *Department of Comparative Pathology and Tropical Medicine, Schools of Medicine and Public Health, Harvard University.*

HELMINTHS FROM THE NORWAY RAT IN NORTHEASTERN OHIO

In the winter and spring of 1940–41 a survey was made of helminthic parasites harbored by Norway rats, *Rattus norvegicus,* in the vicinity of Kent, Portage County, in northeastern Ohio. From twelve different locations, including markets, homes, dumps, barns, slaughter houses, and warehouses, 50 rats were trapped alive and then chloroformed for examination. Identification was confirmed on preserved adult worms with the assistance of the keys of Yorke and Maplestone (1926, Nematode Parasites of Vertebrates) for the nematodes, and of Meggitt and Subramanian (1924, Burma Res. Soc. **17**: 190–237) for the tapeworms.

Helminths were found in 49, or 98% of the rats examined, representing three species of cestodes and four of nematodes: *Hymenolepis nana* (Siebold, 1852) in 26%; *H. diminuta* (Rudolphi, 1808) in 28%; the larval form *Cysticercus fasciolaris* (Rudolphi, 1808) in 10%; *Trichosomoides crassicauda* (Bellingham, 1840) in 42%; *Nippostrongylus muris* (Yokogawa, 1920) in 4%; *Ganguleterakis spumosa* (Schneider, 1866) in 42%; and *Syphacia obvelata* (Rudolphi, 1802) in 4% of the rats examined.

The number of specimens per rat of *H. nana* varied from 1 to 12; of *H. diminuta* from 1 to 4; of *C. fasciolaris* 1 and 2; of *T. crassicauda* from 2 to 20, averaging 7.3; of *N. muris* 8 and 20; of *G. spumosa* from 1 to 58, averaging 15. For *G. spumosa* the ratio of males to females was 2.7 : 1.7; 57% of the specimens were in the colon, and 43% in the cecum, with one specimen aberrant in the kidney of one rat and one in the spleen of another. Of the two cases of *S. obvelata,* one rat had 58 females and 2 males, and the second rat two females only in the colon.

Multiple infections were common, one rat harboring five of the seven species found, lacking only *N. muris* and *S. obvelata;* in three rats four species were found, in two of which were *H. nana, H. diminuta, T. crassicauda,* and *G. spumosa,* and in the third, *H. nana, T. crassicauda, G. spumosa,* and *S. obvelata;* in four rats three species were found, in different combinations including the species found except *N. muris;* of twenty-one rats harboring two species, six had *H. diminuta* and *G. spumosa,* and five had *H. nana* and *T. crassicauda;* and in nineteen rats only one species was found, in nine of which it was *G. spumosa.*—William C. Forbes, *Kent State University, Kent, Ohio.*

THE LIFE CYCLE OF THE TREMATODE *ECHINOSTOMUM CALLAWAYENSIS* BARKER

Considerable numbers of echinostome metacercariae, averaging 0.13 mm in diameter, were found encysted in specimens of *Planaria* being prepared for whole

mounts. When fed to laboratory rats these metacercariae developed in 13 days into mature trematodes identified as *Echinostomum callawayensis* Barker (1915, J. Parasitol. 1: 184–197), described originally from material obtained from the intestine of the muskrat. The only essential difference between Barker's specimens and those reared in rats was in the presence of fewer eggs in the latter.

The first intermediate host proved to be the snail *Physa gyrina*. Naturally infected snails, and also *Planaria*, were obtained from a lake in Ramsey County, Minnesota, where muskrats are abundant. Rediae were found to be of the echinostome type having a short gut and producing cercariae with two rows of about 45 spines. The body of the cercaria measures about 0.30 mm long by 0.18 mm wide, the suckers are subequal, 0.050 to 0.055 mm in diameter, and the tail is approximately 0.32 mm long.

Cercariae are shed in the evening and live for about 24 hours in laboratory containers. When they come in contact with *Planaria* they actively crawl over the surface of the animal, constantly trying the surface. Penetration requires several hours and was repeatedly brought about on uninfected hosts. When fed to rats the cysts produced mature flukes. Tadpoles and guppies could not be infected with the metacercariae.

Aid in the study of the metacercaria was given by Eugene Breault, a student. Details of the life cycle will be published in a subsequent paper.—Ralph W. Macy, *College of St. Thomas, St. Paul, Minnesota.*

AMERICAN SOCIETY OF PARASITOLOGISTS

PRELIMINARY ANNOUNCEMENT OF THE EIGHTEENTH ANNUAL MEETING

Monday, Tuesday, and Wednesday, December 28–30, 1942

New York, New York

In accordance with the action of the Council, the American Society of Parasitologists will convene for a three-day program in conjunction with the meeting of the American Association for the Advancement of Science in New York, New York. The Commodore Hotel has been designated as official headquarters of the Society. The regular meetings will be held in Hunter College, 68th Street and Park Avenue, New York.

The program will be arranged in the same manner as in previous years, with all of the first and third days devoted to contributed papers. The address of the retiring president, Dr. Henry E. Meleney on the subject, "The Role of Parasitologists in the Second World War," will be delivered at the conclusion of the morning session of the second day. The annual luncheon and business meeting will follow the presidential address, and the demonstration program and tea will be held in the afternoon. The demonstration-tea is planned to serve both as a scientific program and as a social function, and to supply the opportunity for informal discussion that is not possible at the regular scientific sessions.

The call for papers has already been sent to members of the Society. In order that the abstracts be published and mailed to the membership before the meetings, it is necessary that they be received in the office of the secretary not later than October 17, 1942. Members are hereby reminded of this date, and are requested to be prompt in the submission of papers for the program.

James T. Culbertson, *Secretary*